THE RISKS AND CONSEQUENCES OF VERTICAL TRANSMISSION OF HIV

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In memory of John, Claire, Danielle, Tammy, Thomas, and Charmaine, and for all those referred to by number but known by name.

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ABSTRACT

The aims of this study were to determine the risk of transmission of HIV from mother to child, to determine factors affecting this risk, to describe the natural history of HIV infection in children, and to evaluate available therapy. Sixty eight infants of HIV seropositive women followed prospectively for a median of 44 months, were studied together with 30 controls, and 8 other children known to be HIV infected.

Sixty one children became HIV antibody negative at 6-18 months. Of 11 HIV infected children, all 10 surviving beyond 18 months remained HIV antibody positive. HIV core antigen was positive in 9/11. HIV was isolated in culture of samples from 9 children; in all 10 cases tested, PCR was positive.

56 index children were followed from birth to over 18 months: 3 were HIV infected, giving a rate of 5.4% (95% CI 0.12-14.87%). Early clinical indications of HIV infection included recurrent respiratory infections, recurrent diarrhoea, eczematous skin rashes, generalised lymphadenopathy, and hepatosplenomegaly. Persistent severe candidal infection heralded the development of AIDS in one infant. HIV infected children were more likely to have persistent hypergammaglobulinaemia, CD4 lymphopoenia and thrombocytopoenia. In the first 2 years, only the difference in IgG levels, noted by 6 months of life, reached significance.

Of 70 infants and their mothers, 5/9 pregnancies within a year

of seroconversion resulted in HIV infected children compared with 7/61 in subsequent years ($p<0.001$). Increased risk of transmission was also associated with advanced maternal disease and CD4 count $<300 \times 10^6/l$ at delivery. Women who bore infected children were more likely to progress to CDC stage IV during follow-up ($p=0.032$).

55% (95% CI 23.38-83.25%) of HIV infected children had symptoms by a year of age, and 82% (95% CI 48.22-97.72%) by 2 years. Median time of progression to stage P2 B-D was 45 months. Mortality was 9% at 1 year (95% CI 0.23-41.28%), 27% at 5 years (95% CI 6.02-60.97%). 6/11 children died at median age of 55 months (95% CI 23.38-83.25%), range 4-82 months. The longest survivor is 76 months. Clinical features associated with progressive disease included PCP, recurrent bacterial infections, LIP, encephalopathy, oesophageal candida, Ewing's sarcoma, cardiomyopathy, and wasting syndrome. Rapid diagnosis of PCP from nasopharyngeal secretions was shown to be possible. The new guidelines for PCP prophylaxis would not have protected one child in our study. Encephalopathy was associated with a poor prognosis, whereas LIP was compatible with prolonged survival. High levels of HIV antigen (>50pg/ml) in the absence of detectable core antibody were associated with progression of HIV disease and mortality within 2 years, as were rapidly declining CD4 counts and raised IgA levels.

Swabs were cultured for respiratory viruses every 3 months from 5 HIV infected and 45 uninfected children. HIV infected children had respiratory symptoms on 14/30 occasions compared with 41/220 for uninfected children ($p<0.01$). On 10/30 and 32/220 occasions

respectively, respiratory viruses were isolated. Respiratory infections were more likely to lead to hospital admissions in the HIV infected children.

Blood from 53 infants was typed for HLA-A, B, and DR antigens. There were no absolute differences in antigenic specificity between HIV infected and uninfected children. However, the combination A3, B7, DR2, only found in uninfected babies, was four times commoner in the study population relative to controls. HLA A1, B8, DR3 haplotype was found less often than expected, but was disproportionately represented amongst infected children.

In eight children receiving intravenous immunoglobulin infusions every 3 weeks. Significant improvement was noted in terms of weight gain, number of infective episodes, and days spent in hospital. This resulted in a 49% saving in terms of cost. HIV core antigen was detected in 5 children prior to treatment but was absent 6 months later. CD4 counts continued to fall. Six children also received zidovudine. There was a significant improvement in weight gain, and in encephalopathy following treatment, together with a reduction in the rate of decline of CD4 count. This intervention may have affected the natural history of HIV infection in the study children.

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The following papers based on the work presented in this thesis have been published:

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The European Collaborative Study (1992). Risk factors for mother-to-child transmission of HIV-1. *Lancet*; 339: 1007-1012.

ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ARC	acquired immunodeficiency syndrome related complex
ARV	AIDS related virus
AZT	zidovudine, azidothymidine
CD4 count	CD4+ lymphocyte count
CDC	Centers for Disease Control, Atlanta, Georgia, USA
CI	Confidence interval
CMV	Cytomegalovirus
CNS	Central Nervous System
CSA	Common Services Agency, Lothian Health Board, Edinburgh
CT	computerised tomography
dATP	deoxyadenosine triphosphate
ddC	2',3'-dideoxycytidine
ddI	2',3'-dideoxyinosine
dGTP	deoxyguanine triphosphate
dCTP	deoxycytosine triphosphate
dTTP	deoxythymidine triphosphate
DNA	deoxyribosenucleic acid
DT	diphtheria/tetanus vaccine
DTP	diphtheria/tetanus/pertussis vaccine
EBV	Epstein Barr Virus
ECG	electrocardiograph
ECS	European Collaborative Study of vertical transmission of HIV
EDTA	ethanyl diamine tetracetic acid
ELISA	enzyme linked immunoassay
FCS	foetal calf serum
GP	General Practitioner
HIV	human immunodeficiency virus
HIV Ab	HIV antibody
HIV Ag	HIV p24 core antigen
HLA	human leucocyte antigen
HSV	herpes simplex virus
HTLV III	human T lymphotrophic virus type III
ID	infectious disease
IQ	intelligence quotient
ITP	immune thrombocytopoenic purpura
IV	intravenous
IVAP	in vitro antibody production
IVIgG	intravenous immunoglobulin
KS	Kaposi sarcoma
LAV	lymphadenopathy associated virus
LIP	lymphocytic interstitial pneumonitis
MCV	mean corpuscular volume
MMR	measles/mumps/rubella vaccine
MRI	magnetic resonance imaging
mths	months
ND	not done
neg	negative
NICH	National Institute of Child Health
no	number

NS	not significant
OD	optical density
OR	odds ratio
p24 Ab	HIV anti p24 core antibody
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCP	Pneumocystis carinii pneumonia
PCR	polymerase chain reaction
Paed	paediatrician
Pos	positive
RNA	ribose nucleic acid
RPMI	growth medium produced by Rose Park Memorial Institute
RSV	respiratory syncytial virus
SIDS	sudden infant death syndrome
TID	three times daily
TNF	tumour necrosis factor
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
UV	ultraviolet
WHO	World Health Organisation
ZIG	Zoster immune globulin

INTRODUCTION

In the 9 years since the first case of AIDS in the UK was recognised in 1982 (Editorial, Br Med J 1983), there have been over 5,000 cases reported, 314 of which were in women, and 49 in children. In Scotland, there have been 277 cases, 33 of whom were women and 5 in children; 4 of these have already died (Communicable Diseases Scotland Weekly Report 1991). In England and Wales, most cases come from the homosexual/bisexual population, whereas in Scotland intravenous drug users make up around 70% (Bannister et al 1988). This pattern is more similar to that seen in Southern Europe.

In Edinburgh, there was an epidemic of intravenous drug misuse in the early 1980s consequent upon the increased availability of heroin at this time. In 1982 there was an outbreak of hepatitis B infection among users, attributed to an increase in sharing needles owing to an acute shortage caused by the closure of the local legal supplier. Thus when HIV was introduced into this population in 1983, it spread rapidly through the drug- using population (Robertson et al 1986 [a]). The earliest known seroconversion was in January 1983 in a person recently having returned from Southern Europe, who went on to share with users in Edinburgh (Bisset et al, 1989), the majority of whom seroconverted later that year and in early 1984 (Robertson et al, 1986 [a]). Seropositive rates of between 38% and 65% in this group have been reported (Peutherer et al 1985, Robertson et al, 1986 [a], Brettle et al 1986). This contrasts sharply with rates of below 5% in Glasgow and London (Follett et al 1986, Webb et al 1986), and the difference is largely attributable to the difference in

needle sharing practices (Robertson et al 1986 [b], Brettle 1986).

The prevalence among the drug using population had implications for the further spread of the epidemic. Not only were they a mobile group, sharing in other cities and countries (Brettle et al 1987, Bisset et al 1989), opening the possibility of similar epidemics elsewhere, but they were young and sexually active, and so provided a channel for further spread into the non-drug using heterosexual population (France et al 1988). Also a third were women of child bearing age (Brettle et al 1987), who from early in the epidemic were becoming pregnant, with the risk of transmission to their children (Robertson & Bucknall 1986). The impact on maternal and child health, and the counselling given to infected women wanting children or already pregnant, would therefore be dependent on the rate of vertical transmission, and the natural history of the disease in those infected. At the outset of the study, there was much debate as to whether termination of pregnancy should be advocated for all pregnant women who were seropositive for HIV because of the risks to the mother and child.

Following the recognition of the high rates of HIV infection among intravenous drug users in Edinburgh (Peutherer et al 1985, Brettle et al 1986), HIV seropositive women who were either pregnant or who had recently given birth began to seek medical attention and advice, regarding the risk of their child being infected with HIV and the likely outcome for the child should this prove to be the case. At this time, the risk of transmission to their offspring was felt to be high (Minkoff et al 1987), but results from prospective studies were not available. Centres which

reported high rates of vertical transmission of HIV had initially concentrated on women who had more advanced disease, and a large proportion of those studied were black or Hispanic. The same risk might not apply when the mother was Caucasian and had preclinical disease. As the first cases were described only 3 years previously, and would therefore represent children more severely affected, many questions remained unanswered regarding the spectrum and natural history of HIV disease in children. Guidelines did not exist for managing children born to HIV seropositive women, and therapeutic options were at an early experimental stage.

We therefore set out to identify women who were HIV antibody positive and who had recently had infants or who were in various stages of pregnancy. On 1 January 1986, a clinic was established, which not only provided paediatric advice and medical care for these infants, but also formed the setting for the study of perinatal transmission of HIV in Edinburgh.

The aim of this study was to answer the following questions:

a) What is the exact risk of virus transmission from an HIV positive mother to her infant?

b) Are there identifiable genetic, maternal, or perinatal factors affecting the transmission of HIV from seropositive mothers?

c) What are the features which most readily identify those infants and children who are infected with HIV?

d) What is the natural history of the clinical, immunological and virological abnormalities in infants and children born to HIV positive mothers?

e) Which (if any) of the parameters investigated give the earliest prognostic indicators for disease progression in the infected children?

f) What are the therapeutic options available, and what is their role in the management of the infected children?

g) What is the long term outlook for those children who are i) infected ii) presumed uninfected?

SECTION I

REVIEW OF THE LITERATURE

1 Epidemiology of AIDS

The Acquired Immunodeficiency Syndrome was first described in mid 1981 when 5 cases of Pneumocystis carinii pneumonia in previously well homosexual men in Los Angeles, and 26 cases of Kaposi's sarcoma in New York and Los Angeles were reported to the CDC. (CDC 1981 [a], CDC 1981 [b]). All these patients had in common an acquired defect in cell mediated immunity which predisposed them to rare tumours and opportunistic infections (Gottlieb et al 1981, Masur et al 1981, Siegal et al 1981, Hymes et al 1981). However, it is now recognised that sporadic cases occurred before this time, as early as 1959 (Nahmias et al 1986, Corbitt et al 1990, Huminer et al 1987, Froland et al 1988). Although the first cases reported were in homosexual or bisexual men, a similar spectrum of disease was found to involve haemophiliacs (CDC 1982 [a]) and transfusion recipients (CDC 1982 [b], Curran et al 1984). Intravenous drug users (one third of whom are women) were also implicated (CDC 1983 [a], Ginzburg 1984), and also heterosexual partners of those with or at risk from AIDS (CDC 1983 [b], Pitchenik et al 1983, Harris et al 1983). This raised the possibility of spread among sexually active women of child-bearing age, and therefore of spread to their offspring. Affected infants and young children were soon discovered, some of whom had received blood products (Ammann et al 1983), but in the majority, their mothers were in high risk groups (CDC 1982 [c], Ammann 1983, Joncas et al 1983, Oleske et al 1983, Buck et al 1983, Thomas et al 1984, Rubinstein

et al 1983, Scott et al 1984, Cowan et al 1984, Vilmer et al 1984).

At this time the aetiology of the syndrome was unknown. The epidemiology of AIDS suggested that its transmission was akin to hepatitis B, through blood and blood products, and sexual contact, and so the search for a transmissible agent, most likely to be a virus, commenced. In 1983, a type C RNA retrovirus tropic for T lymphocytes was isolated from a patient with symptoms and signs preceding AIDS (Barre-Sinoussi et al 1983). This virus was variously called the Human T-cell Lymphotrophic virus Type III (HTLV III), AIDS Related Virus (ARV) and Lymphocyte Associated Virus (LAV) (Barre-Sinoussi et al 1983, Broder & Gallo 1984), and could be cultured from peripheral blood lymphocytes, lymph nodes, semen, and saliva of patients with either ARC or AIDS (Gallo et al 1984, Zagury et al 1984, Ho et al 1984, Groopman et al 1984). Over 90% of these patients were found to have antibody to the virus, compared with less than 1% of healthy individuals or patients with other immunosuppressive diseases (Gallo et al 1984, Sarngadharan et al 1984). In May 1986, the International Committee on the Taxonomy of Viruses agreed that the virus should be called HIV - the human immunodeficiency virus.

Throughout the world, three broad patterns of HIV have been described by the WHO (Chin & Mann 1988). In North America, Western Europe and Oceania, the primary groups affected are homosexual men and intravenous drug users; extensive spread of HIV began in the late 1970s/ early 1980s. There are not many paediatric AIDS cases in these areas as heterosexual spread, although increasing, accounts for a relatively small number of new infections. However, in sub-Saharan

Africa and some parts of the Caribbean, extensive heterosexual spread began in the mid- to -late 1970s, and so perinatal transmission of HIV is a major problem. HIV was introduced into Asia, Eastern Europe, North Africa and the Middle East in the early to mid 1980s, and although spread is occurring, there is no clearly predominant mode of transmission, and the prevalence remains low. However, in some countries this situation is changing rapidly (Chin 1990). In Eastern Europe, the incidence of perinatal transmission may be low, but spread has occurred extensively among children in hospitals and other institutions through repeated use of needles, where disposable equipment is unavailable (Anon, Wkly Epidem Rec 1990, Patrascu et al 1990, Rudin et al 1990).

The incidence of AIDS is increasing worldwide, with more than 263,000 cases from over 160 countries reported to the World Health Organisation. Nearly half of these cases were reported to the Centers for Disease Control from the USA. Cases from sub-Saharan Africa are probably under-represented due to difficulties in reporting, and so this figure grossly underestimates the true total (WHO Global Statistics 1990). The WHO estimates that during the first decade of the HIV pandemic there were about 500,000 cases of AIDS in women and children, most of whom were unrecognised (Chin 1990). As the disease in children carries a 5 year mortality of 80%, the medical, public health and psychosocial implications are profound.

2 Pathogenesis of HIV

HIV belongs to a sub-family of human retroviruses known as lentiviruses, characterised by possession of a large genome, containing several viral genes, the ability to induce cytopathic effects in infected cells, causing disease with a long incubation period, and resulting in immunological disorders and neurological disease (Levy 1986). The basic structure of the virus is shown in fig I.2.1. Infection of T lymphocytes bearing CD4 is well recognised, but HIV can be cultured from macrophages, microglia and oligodendrocytes in the brain, and other cells in the skin, gastrointestinal tract, and elsewhere (Levy 1989). The life cycle (fig I.2.2) begins with attachment to a specific cell surface receptor, commonly the CD4 molecule. Fusion of viral and cell membranes then occurs, releasing core virion into the cytoplasm. Viral reverse transcriptase synthesises double stranded DNA, which integrates into the cell chromosome. Then follows a latent phase, where there is little or no expression of viral gene products. The final phase of the cycle is initiated when viral replication is stimulated. The viral RNA and viral proteins are synthesised, and the progeny is produced at the cell surface by budding.

The subsequent depletion in numbers of CD4+ lymphocytes is not only caused by death of infected cells, but is also due to cell-to cell fusion, forming multinucleated giant cells or syncytia, composed of both infected and non-infected cells (Folks et al 1987). These then develop ballooning cytoplasm and die. Abnormal cellular immunity results not only from the CD4+ lymphocyte depletion, but also by qualitative

defects in T cell function (Bowen et al 1985), and in children, the reduced CD4/CD8 ratio is partly due to an increase in CD8+ (suppressor T) lymphocytes (Oleske et al 1983, Rubinstein et al 1983, Scott et al 1984). In vitro abnormalities in lymphocyte proliferation after mitogen and antigen stimulation can be demonstrated in some HIV infected children, but normal responses are also seen (Rubinstein et al 1983, Ammann & Levy 1986). In addition to CD4+ lymphocyte dysfunction, humoral immunity is abnormal in most HIV infected children (Bernstein et al 1985). Raised serum IgG, IgA and IgM due to polyclonal B cell activation occurs commonly (Ammann 1985, Zolla-Pazner 1986), but panhypogammaglobulinaemia has also been described (Maloney et al 1987, Pahwa et al 1987, Espanol et al 1987). Even when serum immunoglobulin levels are raised, there is impaired antibody production (Lane et al 1983). It is likely that other direct and indirect effect of the virus contribute to the immunopathology. The immunological response of the host may result in cytotoxic T cells attacking both virus-infected and uninfected cells (Siliciano et al 1988). Production of autoantibodies may also contribute to some disorders, such as immune thrombocytopoenic purpura.

Neurological disease is common in AIDS, both in adults and children. In the context of immunodeficiency, CNS infection due to conventional and opportunistic pathogens might be anticipated, which together with CNS lymphoma and cardiovascular accident are found in 15% of children with neurological deficit (Belman et al 1988). The majority, however, develop primary HIV encephalopathy. It is likely that migration into the brain of HIV infected macrophages occurs early (Wiley et al 1986), and infection of brain endothelial and glial cells may occur in

the majority of patients. After a latent period, reactivation and expression of HIV antigens may lead to the inflammatory cell invasion of the CNS which has been observed. Macrophages probably carry additional virus into the brain and lead to further dissemination (Epstein, et al 1988 [a]). HIV infected macrophages also produce TNF, which has been demonstrated to be toxic to oligodendrocytes in vitro (Selmaj & Raine 1988). Elevated serum levels of TNF have been shown to be associated with progressive encephalopathy in children with AIDS (Mintz et al 1989), and it may that TNF provides the link between HIV-1 infection, inflammation, and the white matter damage seen in progressive encephalopathy.

The similarity between the myelopathy associated with HIV infection and subacute combined degeneration due to vitamin B_{12} deficiency has led to the search for disorders of methylation within the CNS. HIV infected children with neurological complications have low CSF S-adenosylmethionine concentrations which could be the result of interference with folate metabolism limiting the availability of methyl groups for myelin synthesis (Surtees et al 1990). The low methylation ratio is found even in patients who are B_{12} and folate replete, and may occur from a very early stage of HIV infection (Keating et al 1991).

Other strains of HIV directly infect the epithelial cells in the bowel and HIV infected lymphocytes and macrophages may also be found within the gut wall (Editorial, Lancet 1989), resulting in chronic diarrhoea and malabsorption. Mucosal immunodeficiency may also lead to bacterial overgrowth of the gastrointestinal tract, with alteration in the normal

flora (McLoughlin et al 1987). As is the case in CNS disease, toxic cytokines produced by macrophages may contribute to gastrointestinal symptoms (Levy 1989).

FIGURE I.2.1
Structure of HIV

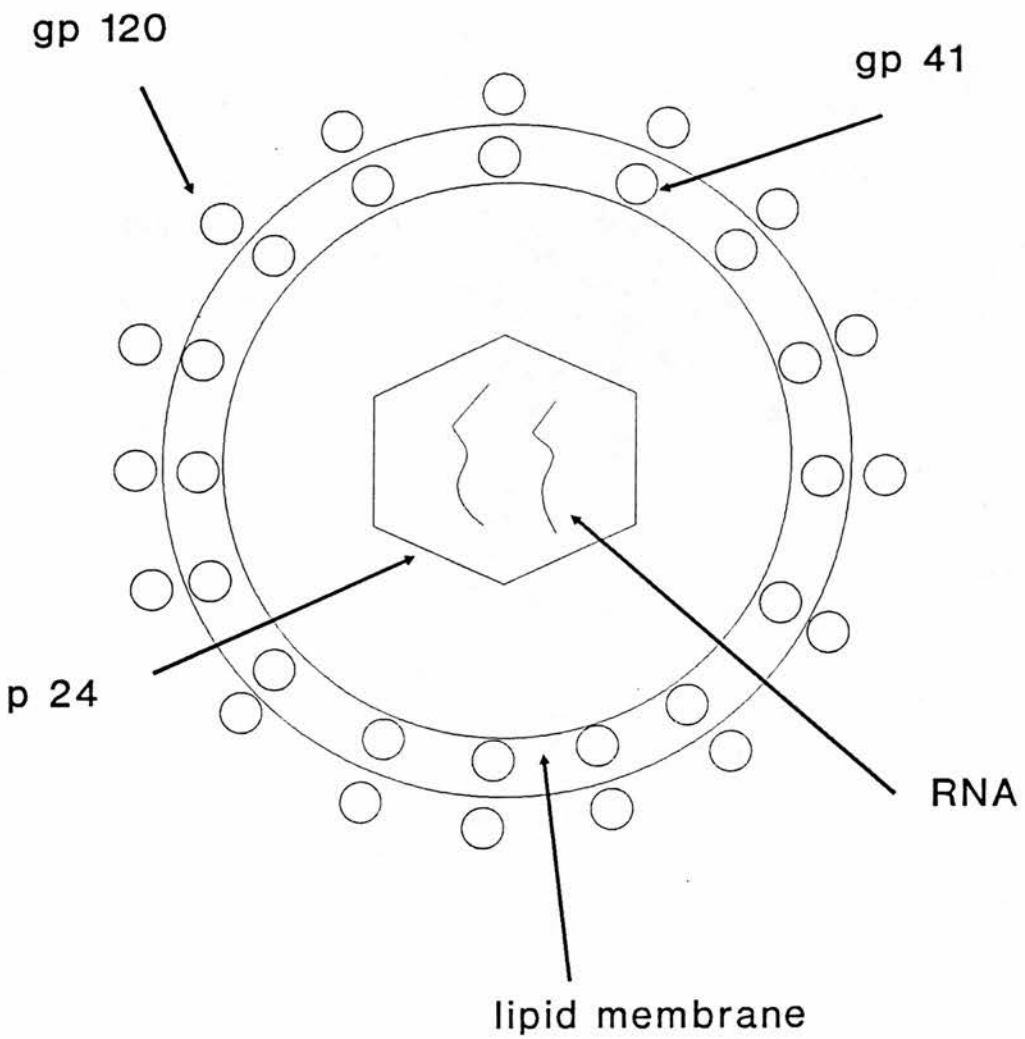
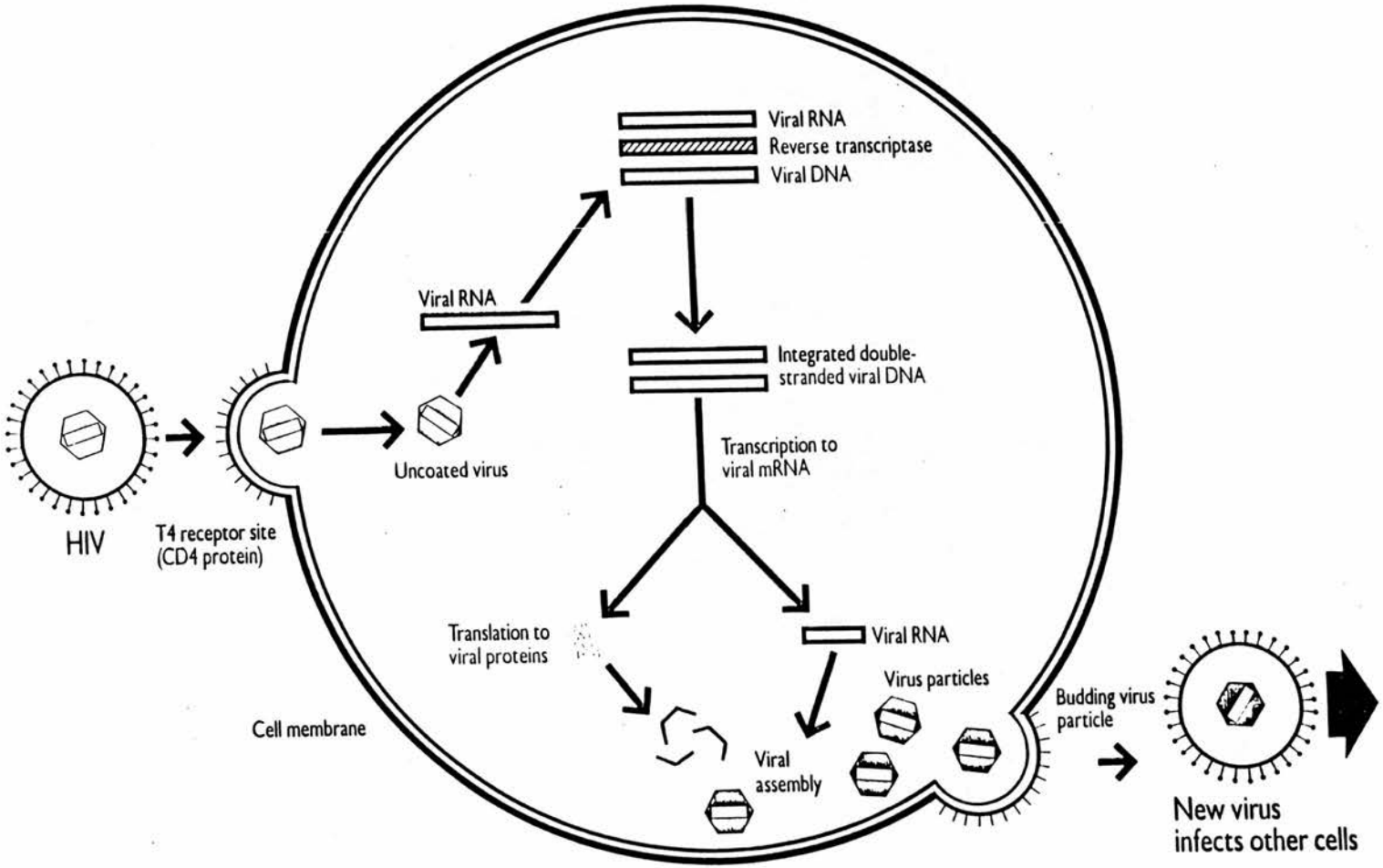


FIGURE I.2.2



Schematic illustration of HIV replication

3 Laboratory diagnosis of HIV infection

In 1985 an enzyme linked immunosorbent assay (ELISA) using disrupted whole HIV virus as the antigen was developed to detect antibodies to HIV (Weiss et al 1985), and is the basis of screening. Despite the high level of sensitivity and specificity of the available ELISAs (Reesink et al 1986), problems with false positives will occur when screening a low risk population (Sivak & Warmser 1985). A positive result must therefore be verified by being repeatedly reactive, and by another test, such as Western blot, or immunofluorescence assay must also be positive (CDC 1987 [a]). Western blots of sera from the majority of infected individuals display bands corresponding to most of the major HIV structural proteins, and for a test to be unequivocally positive, antibodies must be reactive with multiple bands, ie, p24, p31 and either gp41 or gp160. If fewer bands are present, the test is considered indeterminate (Busch 1988). Only if no bands are present is the test considered negative. An indeterminate pattern in ELISA negative individuals is common, however, and does not correlate with the presence of HIV 1 (Genesca et al 1989). The place of the Western blot as the 'gold standard' for other assays has therefore been increasingly disputed (Mortimer 1991).

Even if an ELISA with 100% sensitivity were developed, there would still be a 'seroconversion window' following exposure to the virus before production of detectable antibody. In most adults, exposure to the virus is followed within 3-6 weeks by prolific replication of virus. Antibodies can be detected within 2 or 3 months, and the level of circulating virus falls subsequently (fig I.3.1). Antibody levels detected

by ELISA and Western blot remain high thereafter. With disease progression, antibody to core antigens may decline, and the concentration of virus in the plasma rises (Allain et al 1987). However, two other patterns have been described (Haseltine 1989). Very rarely, a previously seropositive person may lose antibodies detectable by ELISA and Western blot (fig 1.3.2), but proviral DNA may be detected in lymphocytes by PCR (Loche & Mack 1988). Fig 1.3.3 shows a third pattern where it is possible for the infected person to remain seronegative for a prolonged period of at least 35 months following initial infection (Imagawa et al 1989). As yet there have been no reports of vertical transmission occurring during this period and the degree of infectivity remains unknown. It must be emphasised that patterns 2 and 3 are uncommon, and in a study, modelling cases of HIV infection with known exposure in published reports, the median time from exposure to antibody detection was 2.1 months, 95% of cases seroconverting within 5.8 months (Horsburgh et al 1989).

In children born to HIV seropositive women, the diagnosis of HIV is complicated by the presence of transplacentally acquired maternal HIV antibody which may take up to 18 months to clear (ECS 1988), and also by the more unpredictable response of the immature immune system to HIV infection. Positive HIV antibody is therefore is only useful diagnostically after this age. HIV infected children who are negative for HIV antibody have also been described(Pahwa et al 1986, Borkowsky et al 1987). Anti HIV IgM testing is of low sensitivity and specificity (Parry & Mortimer 1986). Further information has been sought by looking at the pattern of anti HIV IgM and IgG sub-classes in children at risk of

HIV infection (Kwang et al 1987). An alternative to these methods has been to demonstrate in-vitro production of HIV specific IgG by peripheral blood lymphocyte cultures from children at risk of infection (Amadori et al 1988).

Assays for the direct detection and quantification of specific HIV antigen (primarily HIV core protein)have been developed and HIV Ag can be detected in the plasma of HIV infected individuals (Lange et al 1986). Studies in adults have shown that in primary infection, detection of HIV Ag may precede HIV antibody (von Sidow et al 1988), although this is not such a consistent feature of asymptomatic seroconversion (Allain et al 1987). Subsequent disappearance of HIV Ag may be due to a low level of virus replication or to the formation of immune complexes after the initial antibody response. Reappearance of detectable HIV Ag together with a decline in HIV anti-core antibody levels is associated with progression of clinical HIV disease (Allain et al 1987). A larger proportion of asymptomatic children than adults have persistent antigenaemia (Borkowsky et al 1989). While this might reflect a different balance between active and latent HIV infection in children (Lange et al 1986), persistent antigenaemia has been reported to be associated with disease progression and a poor prognosis (Epstein et al 1988 [b]). Although cross sectional studies have now been undertaken (Ellaurie & Rubinstein 1991), at the outset of our study no reports were available on the course of HIV Ag and p24 Ab levels in children over periods longer than 2 years for correlation with the progression and clinical symptomatology of HIV infection.

Because of the limitations of serological diagnosis, methods have been developed to detect virus or viral components. HIV can be isolated from peripheral blood mononuclear cells (Griffith 1987). Since their initial development, techniques have improved, so that in a recent study of over 100 seropositive subjects, HIV was isolated from 100% of symptomatic, and 87% of asymptomatic patients, with all 85 seronegative controls proving culture negative (Ulrich et al 1988). In children, a positive result is useful. but HIV cannot be isolated from the peripheral blood of all infected children (Cowan et al 1988).

The polymerase chain reaction is an in vitro method of selectively amplifying specific DNA sequences (Saiki et al 1985, Saiki et al 1988). It has been used successfully to detect the presence of HIV pro-viral DNA in the peripheral circulation of HIV infected individuals (Kwok et al 1987, Ou et al 1988). In adults, PCR may allow detection of HIV infection prior to seroconversion (Hewlett et al 1988) and has been reported to detect latent seronegative infection (Farzadegan et al 1988, Pezzella et al 1989, Imagawa et al 1989). The application of PCR has also enabled the earlier diagnosis in children (Laure et al 1988, Rogers et al 1989, Edwards et al 1989). This technique still requires further evaluation, however, as it is at present a research tool with problems of contamination and standardisation.

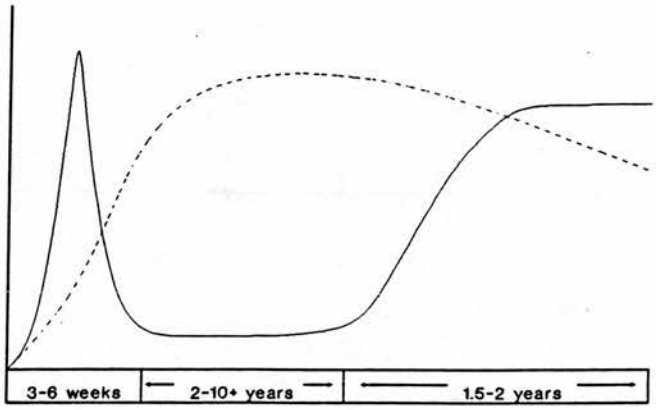
Other laboratory abnormalities, such as hypergammaglobulinaemia or abnormal CD4 count may also suggest the diagnosis of HIV infection in children at risk (CDC 1987 [b]). The European prospective study indicates that raised IgG, A, or M at 6 and 12 months of age is 77% sensitive, and 97% specific for HIV infection.

Although the CD4/CD8 ratio is specific, at this young age it lacks sensitivity (ECS 1991). At present, the infection status of children under 15 months who retain maternal antibody but have no other evidence of infection remains indeterminate (CDC 1987 [b]). Prospective follow-up of children to determine what are the early symptoms, signs and laboratory criteria most indicative of infection is therefore required.

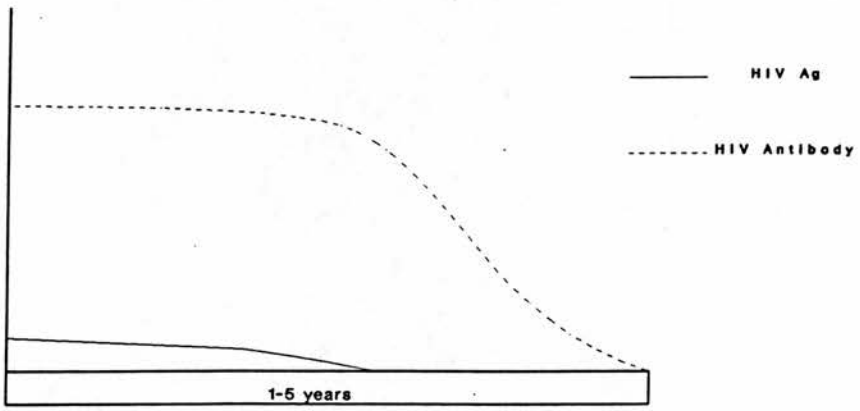
FIGURES I.3.1-3

Patterns of HIV antigen and antibody with time

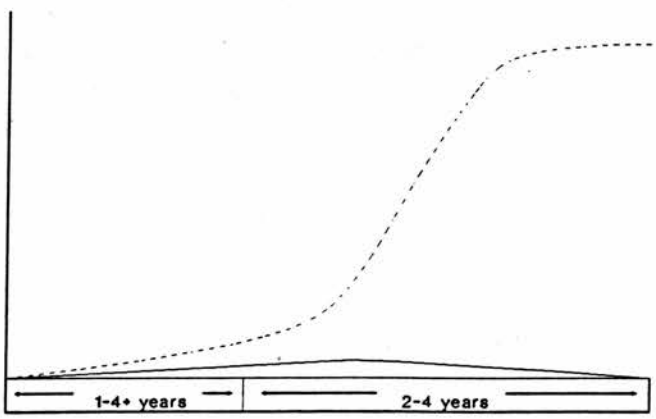
1



2



3



4 Vertical transmission of HIV

In the majority of HIV infected children, the virus has been transmitted from mother to child (Rogers et al 1987). By the end of 1990, 83% of all AIDS cases less than 13 years of age had mothers at risk of HIV infection (WHO 1990). Five percent were haemophiliacs, and a further 10% had received blood products, but screening of blood has now reduced almost to zero the incidence of new cases transmitted in this way. The continuing risk to the paediatric population is therefore largely through vertical transmission.

Although vertical transmission of HIV is now well documented (Sprecher et al 1986, Lapointe et al 1985, Jovaisas et al 1985), the exact risk has not yet been quantified. Rates of transmission appear to vary from 6.9-65%, depending on the inclusion criteria and duration of the study, as well as methods used to define paediatric HIV infection (Mok et al 1987, Mok et al 1989 [a], Blanche et al 1989, ECS 1991, Minkoff et al 1987). Higher rates were reported in early studies when identification of cases depended on the observation of clinical disease in the mother or affected sibling (Scott et al 1983), but variation still remains in prospective studies (Mok et al 1989 [a], ECS 1991, Ryder et al 1989).

Previous studies have suggested that pre-term labour (Goedert et al 1989), chorioamnionitis (Maynard et al 1990), and advanced maternal disease (Hira et al 1989, Ryder et al 1989) may increase the risk of transmission, but clear evidence is lacking. High titre antibodies in maternal serum to portions of the envelope glycoprotein gp120 are reported to be protective by

some groups, (Goedert et al 1989, Rossi et al 1989, Devash et al 1990) but others have failed to substantiate these findings (Parekh et al 1991). At the time of this study there were no reports relating time of maternal seroconversion to risk of transmission.

It has been suggested that HIV infected individuals are more infective at the time of seroconversion and in the later stages of the natural history of HIV infection (May 1988, Laga et al 1989, European Study Group 1989). This could well be true of vertical as well as for heterosexual transmission. Furthermore, although it was initially suggested that pregnancy was a factor possibly leading to a more rapid progression of HIV disease (Schoenbaum et al 1987, Biggar et al 1989), subsequent data has suggested this not to be the case (Selwyn et al 1989, Maynard et al 1990). There is little long term post-delivery data on the effect of bearing an infected child on prognosis for its mother.

The variation in transmission rates reported between different cohorts, and the variable course of HIV disease may in part be a reflection of individual differences in genetic susceptibility. Several studies have associated HLA specificities with aspects of HIV infection. Examples include HLA-B35 and progression from lymphadenopathy syndrome (CDC stage III) to AIDS in subjects infected predominantly from intravenous drug use (Smeraldi et al 1986, Smeraldi et al 1988); HLA-DR5 with HIV-associated Kaposi's sarcoma (Pollack et al 1983, Prince et al 1984) and thrombocytopoenia (Raffoux et al 1987) in homosexuals; and HLA-DR3 with disease progression in haemophiliacs (Steel et al 1988, Kaslow et al 1990, Fabio et al 1990) but not in a predominantly homosexual population where the

opposite trend was apparent (Kuntz Bruster 1989). In addition to individual specificities, the combination or haplotype HLA- (A1, B8, DR3) has been associated with susceptibility to infection and relatively rapid loss of circulating CD4+ lymphocytes (Steel et al 1988, Kaslow et al 1990, Cameron et al 1990).

In principle, HLA phenotype might influence disease susceptibility in utero, or, amongst those infected, influence disease progression in infancy. However, children of HIV-infected mothers had not previously been studied in relation to tissue type.

5 HIV infection in children

During the first half of the 1980s, much information about AIDS in children became available, with detailed descriptions of the syndrome by Oleske et al (1983), Scott et al (1984) and Rubinstein et al (1983). Because the syndrome was initially described before the cause was known, the nomenclature can be confusing. The initial requirement by the CDC for a diagnosis of AIDS in children less than 13 years was for a documented opportunistic infection or AIDS associated malignancy in the absence of underlying primary or secondary immunodeficiency (CDC 1984). Lymphocytic interstitial pneumonitis (LIP) was included as a diagnostic criterion in 1985 (table I, Appendix I)(CDC 1985). In 1987, after more experience of HIV infection had been gained. and laboratory techniques for diagnosis improved, the definition was revised (table 2, Appendix I)(CDC 1987 [c]). It is anticipated that this revision will have a substantial impact on future AIDS surveillance trends (Stehr-Green et al 1988), and some children with a previous diagnosis of ARC are now classed as AIDS (Falloon et al 1989). It is therefore important when reviewing data on AIDS in children to know which definition was used. Because of the reliance on the availability of laboratory information for the revised definition, its use is limited in areas such as rural Africa. The WHO has therefore developed a clinical case definition for use in such circumstances (table 3, appendix I)(WHO 1986). In practice, although its specificity is high, the sensitivity and positive predictive value has been found to be low, and further amendments have been suggested(Lepage et al 1989).

Not all children with HIV infection meet the criteria for AIDS. For those who do not, HIV infection cannot be diagnosed on clinical criteria alone (table 4, appendix I) (CDC 1987 [b]). In the early stages when signs and symptoms are non-specific, a diagnosis of HIV infection based on clinical evidence alone is especially unreliable but laboratory tests may also be unhelpful. The child's infection status therefore remains indeterminate. To enable data collection on the incidence of various clinical manifestations, CDC has devised a classification scheme, reproduced in table 5, appendix I. (CDC 1987 [b]). All references to clinical stage of the study children will be made according to this classification.

6 Natural history of paediatric HIV infection

HIV infection may be evident from birth, (Marion et al 1986), and in 1986, 50% of children with AIDS were diagnosed during the first year of life, and 82% by 3 years of age (CDC 1986 [a]). The European Collaborative Study reported in 1991 that by 12 months, 26% have AIDS, and 17% die of HIV-related disease(ECS 1991). The mean age at diagnosis has appeared to increase over time, maybe because those with mild or asymptomatic disease had previously remained undiagnosed. The onset of AIDS has been delayed over 7 years in some children (Kelly et al 1987), but in general, it is thought that the incubation period is shorter in children than adults, and in congenital than transfusion-acquired infection (Rogers et al 1987, Medley et al 1987). Clinical evidence of infection is unreliable in the early stages when signs and symptoms are non-specific (CDC 1987 [b]). The inclusion of control children in prospective studies of children at risk of HIV infection is therefore essential, if clinical and laboratory parameters are to be evaluated. No previous study of mother to child transmission of HIV had included such a control group.

Respiratory symptoms are common in the immunocompromised child, and Pneumocystis carinii is an important pathogen which must be considered in the differential diagnosis. In HIV infected children, it is the chief opportunistic infection, occurring in 29% of AIDS cases reported to the CDC up to the end of 1990 (CDC 1991), and in over 50% if children progressing to AIDS in the first year of life (Oxtoby 1990, Scott et al 1989, Krasinski et al 1989). Characteristic radiological

appearances are of diffuse reticulogranular infiltrates, particularly in the perihilar region, progressing to consolidation, but a normal chest X ray is compatible with the diagnosis (Barter 1987). Cytomegalovirus or mycobacteria infection, candidal pneumonia and bacterial sepsis may produce similar findings. Definitive diagnosis has previously relied on the demonstration of silver staining cysts in material obtained from open lung biopsy or bronchio-alveolar lavage, requiring an invasive procedure in an acutely ill child (Wolff et al 1977, deBlic et al 1987). Sputum induction (Zaman et al 1988) in infants and small children is not a practicable technique, and serology is unhelpful (Williford Pifer et al 1988). Non-invasive methods of identification of Pneumocystis carinii therefore require development.

Although effective antimicrobial therapy exists, the mortality from PCP in infancy remains high (Bernstein et al 1989, Vernon et al 1988). Guidelines for primary prophylaxis for PCP in adults are well established (CDC 1989). However, CD4 counts are normally higher in infants than in older children and adults, and PCP commonly occurs in infants with CD4 counts higher than the $200x10^6/l$ recommended in adults for commencement of primary prophylaxis (Leibovitz et al 1990, Kovacs et al 1991). Different guidelines according to age have therefore recently been produced (CDC 1991). These guidelines are summarised in Table 6, appendix I, and the recommended regimens for chemoprophylaxis in table 7, appendix I. The implications of these guidelines for the management of infants whose HIV infection status is still indeterminate are yet to be explored.

Respiratory infections may also be caused by viruses such as CMV and measles which may cause disseminated infection in these children (Pahwa et al 1986, Rubinstein et al 1986, Joshi et al 1985, Markowitz et al 1988). The natural history of respiratory infections in HIV infected children due to other viruses common in the paediatric population has received little attention, and requires further study.

Children commonly present with recurrent bacterial infections (Scott et al 1984, Bernstein et al 1985 [a]), and defective humoral immunity has been demonstrated (Bernstein et al 1985 [b]). Even in the presence of hypergammaglobulinaemia, patients develop symptoms and signs similar to those suffered by children with primary hypogammaglobulinaemia. Hence passive immunisation in the form of intravenous immunoglobulin has been reported to be of benefit (Calvelli & Rubinstein 1986, Oleske et al 1987). At the time of our study, no controlled trial of treatment with IVIgG had been carried out.

In HIV infected children, serum levels of IgG, IgA and IgM are typically raised (Amman 1985), and can be 3 or 4 times the upper limit of the normal range. It has previously been reported that a polyclonal hypergammaglobulinaemia may be associated with a raised serum viscosity in very rare cases (reviewed in Martin et al 1989), but no report of symptomatic hyperviscosity has previously been documented in HIV infected children.

Present therapeutic options for HIV infected children are otherwise limited to treatment for specific infective episodes(Young 1987),

and to zidovudine. Since zidovudine was shown to have in vitro activity against HIV in 1985 (Mitsuya et al 1985) and later to decrease the frequency and severity of opportunistic infections, and the mortality rate in adults with advanced HIV disease (Fischl et al 1987), much experience on the use of this drug has been gained. In children, early experience based on continuous intravenous infusion demonstrated clinical improvement, particularly in neurodevelopmental abnormalities and in weight gain (Pizzo et al 1988 [a], Blanche et al 1988). Pharmacokinetic studies have shown that continuous infusions are more effective in maintaining a virostatic plasma level of 1umol/l than bolus doses and that on an oral regimen of 720mg/m^2/day in 4 divided doses, this plasma level is maintained for less than half the day (Balis et al 1989 [a], [b]). It is unclear whether this is important clinically, and if zidovudine is to be available to the large number of affected children, an oral regimen is the only practicable method of delivery. Data on the efficacy of this drug when given on a long term basis, and on toxic effects need to be accumulated.

SECTION II

METHODS

1 Organisation of the study

Subjects

The study aimed to identify all infants born to HIV seropositive women within Lothian Region: these infants comprised the index group. Referrals were made by staff in the adult clinics, obstetricians, midwives, neonatal paediatricians, general practitioners, social workers, and health visitors upon discovery of a pregnant woman, or mother and infant, considered to be at risk of HIV infection. As a control group, infants whose fathers were HIV seropositive, but whose mothers remained HIV seronegative were enrolled. These mothers were tested after self-identification of high risk activities, and belonged to similar socio-economic groups as mothers testing positive. As additional control material, cord blood from HIV seronegative women with a history of intravenous drug use was obtained, but these infants were not subsequently followed. From the time of my appointment as research fellow, I saw the women in the antenatal clinics where the purpose and nature of the surveillance was explained, after which maternal consent was sought. I was also present at delivery to collect cord blood, and blood from mother, to examine the infant at birth, and to arrange future visits, either in the clinic, or at home, according to the mother's preference.

The study was coordinated from a paediatric HIV counselling and screening clinic, established in January 1986 and staffed by a consultant paediatrician with an interest in community child health (J Mok), and from October 1987 until February 1990, by myself as research fellow. It was held in conjunction with a similar adult clinic supervised by a consultant in infectious diseases (RP Brettle). It initially operated with two sessions per week, during which a health visitor was present and, at one of the sessions, a dental hygienist also. However, with the appointment of a full time health visitor and myself, and with an increasing proportion of the mothers wishing to be visited at home, a more flexible service developed.

In order to study the natural history of HIV infection a further 6 HIV infected infants born to HIV seropositive women were identified. Two (cases 6 and 7) were born and identified in Tayside were referred to the Edinburgh clinic where their subsequent surveillance and management were undertaken. A family in the west of Scotland (cases 8-10) and one child in Newcastle (case 11) were identified and supplied with IVIgG from the Scottish National Blood Transfusion Service. I visited these children every 3 months, carrying out the same surveillance protocol used for the Edinburgh children. They continued to be under the care of the local paediatrician. I also undertook the surveillance and care of one child (case 13) infected with HIV through a blood transfusion in Edinburgh who was receiving IVIgG, using the same protocol. This child was cared for by Dr. J. Mok and Dr. O.B. Eden.

Surveillance procedure and data collection

All infants were seen at birth, 6 weeks, 3 months, then 3 monthly. Control children were followed for one year, after which the option of further review was given to their parents. Those index children who became HIV antibody negative and remained clinically well were seen 6 monthly between ages 2 and 5, and yearly thereafter. At each visit, a history was taken of the child's health, with particular reference to any infective episodes, and visits to the GP. The health visitor assessed development by means of the Denver Developmental Screening Test, after which the infant was examined. Measurements made included weight, head circumference and length (or height). Nose and throat swabs were taken, and placed together in viral transport medium. Peripheral blood was taken for virology (HIV antibody, antigen, virus culture, and PCR), immunology (immunoglobulins, lymphocyte subsets) and haematology (full blood count with differential and platelet count). Immunisation procedures were discussed, and index children were usually immunised by us, according to the standard time schedule. At that time, DPT or DT was given with polio vaccine at 3, 6 and 9 months, and measles (or latterly MMR) at 15 months. Killed polio vaccine (Salk) was given to the children early in the study, but with further experience of the lack of documented adverse sequelae after the administration of oral polio vaccine, and the difficulty in obtaining Salk vaccine, oral polio vaccine was administered to all infants whose mothers did not show evidence of severe immune compromise.

Data on the outcome of the infants who were indeterminate at the completion of my research fellowship, and on the HIV infected children who continued to be followed in Edinburgh thereafter was supplied by Dr. J. Mok and Dr O.B. Eden (case 13). I have continued to follow case 9 according to the same protocol on a 3-monthly basis.

Whenever possible, the mother was reviewed clinically by an obstetrician (FD Johnstone) and a physician (L MacCallum) during each trimester of pregnancy and at delivery, and blood was tested. Thereafter, she had the option of seeing an adult physician at the same visit as the child. Families seen at home were given a separate appointment for mother to be reviewed every three months. In those infants identified after birth, retrospective review of mother's obstetric notes was undertaken and, with maternal consent, stored maternal serum tested for HIV antibody and antigen. Maternal seroconversion date was calculated as the mid-point between the last HIV seronegative and first HIV seropositive sample available. For those in whom no HIV antibody negative sample could be obtained, the mid-point between the date of first possible exposure to HIV and first HIV seropositive sample was taken.

Further management

When infants developed symptoms and signs, either of HIV or other intercurrent infection, they were referred for review to the paediatric team, either by the general practitioner, or by their parents directly. When required, in-patient facilities were available at the

Regional Infectious Diseases Unit of the City Hospital, Edinburgh, where their care continued to be under our supervision.

Children were treated with IVIgG on the basis of a history of 2 or more episodes of bacterial pneumonia, a 3 month history of recurrent or chronic upper respiratory tract sepsis and/or diarrhoea, or symptomatic thrombocytopoenia, with laboratory confirmation of HIV infection. Infusions were given 3 weekly with 200mg/kg of IVIgG supplied by Scottish National Blood Transfusion Service on a hospital day patient basis according to a protocol originally devised for the treatment of primary hypogammaglobulinaemia (Leen et al 1986), . The first infusion was commenced at a rate of 0.6ml/kg/hr, and in the absence of adverse reactions, this was increased to 2.4ml/kg/hr. This IVIgG preparation consists almost entirely of IgG with a normal distribution of IgG subclasses.

As a pilot study, plasma was obtained by plasmapheresis from donor 11 selected during a previous study by Jackson et al (Jackson et al 1988) for high serum titres of anti p24 and neutralising antibody and treated as previously described. Titres of anti p24, anti p41/gp120 and neutralising antibody, were measured, but not antibody to the hypervariable loop. Plasma was then infused in patients 6 and 8 at a dose of 2ml/kg every 6 weeks initially, increasing to 3 weekly after 3 infusions in case 6.

Zidovudine was given orally to 6 children who developed AIDS defining infections (case 8), neurological disease (cases 11 and 13), or

whose absolute CD4 count declined (cases 1,2,6,). All were commenced on 14 mg/kg/day in 3 divided doses, but with further experience in the literature, changes were made subsequently to a dose range of 300-600mg/m^2/day. At first the intravenous preparation was given orally, but latterly, a paediatric suspension was prescribed.

2 Recording and storage of data

Those infants and mothers identified before birth were entered into the European Collaborative study, and a standard proforma for recording data on these children was used as a basis for data recorded on all the children studied, with some additional information also being recorded on the form. Samples of the forms used are included in appendix IV. All data collected since the initiation of the study in 1986 were entered and stored initially on an Atari computer by me using a programme based on DBase III. Latterly an IBM PC compatible system using Dbase IV was used.

3 Laboratory methods and statistics

Samples were analysed by those colleagues acknowledged above. Peripheral blood counts were measured using an automated Coulter technique, with differential leucocyte count performed from a blood film. From October 1987, lymphocyte subsets were measured by flow cytometry (Becton-Dickinson). Absolute numbers of lymphocyte subsets were calculated using as denominator the absolute lymphocyte count obtained manually from the blood film. Immunoglobulins were determined by laser nephelometry.

Serological tests

HIV antibody was measured using an antiglobulin enzyme immunoassay (Abbott Recombinant HIV 1 EIA) followed by a competitive enzyme immunoassay (Wellcozyme). The first sample obtained from a subject and found to be a positive result was confirmed by Western blot. HIV Ag was measured by quantified enzyme immunoassay (Abbott, UK), and expressed as pg/ml, using the standard supplied by the manufacturer with an assay cut-off of 3pg/ml. P24 Ab was measured using the Abbott HIV 1 anti-core enzyme immunoassay. A quantitative value, expressed as p24 Ab index, was derived by dividing the optical density value of the sample tested by the cut-off value, and subtracting this from 1.0 (1-{OD sample/OD standard serum}). Thus the higher the value, the greater the competition (and amount of p24 Ab) in the sample. Zero values indicate lack of antibody.

Lymphocyte culture for HIV isolation

From October 1987, venous blood specimens were collected in preservative-free heparin, and PBMCs were separated over Ficoll/Hypaque using a standard technique. They were then cultured in RPMI 1640 medium containing 2% penicillin/streptomycin, 10% FCS and 80 units/ml interleukin-2 (Dupont). Cell culture supernates were tested at weekly intervals for 4 weeks for the presence of HIV Ag using an antigen capture assay (Dupont). The levels of p24 Ag attained in culture and the time of appearance were recorded. A sample was considered negative if HIV Ag was not detected after 4 weeks' incubation.

Polymerase Chain Reaction (from P Williams et al 1990)

From September 1988, PBMCs were separated from blood samples collected in EDTA over Ficoll-Hypaque, washed once on normal saline and the cell pellet was stored at -20^{O}C for up to 12 weeks. The DNA from the cell pellets was extracted and 1ug aliquots of DNA (equivalent to 150,000 cells) were used for each PCR. PBMCs from 5ml samples of EDTA anticoagulated donor blood were handled identically to serve as controls. The preparation of cell pellets, DNA extractions and PCRs were performed on donor and paediatric blood samples concurrently, in order to be able to detect any cross-contamination during each experimental stage.

A double PCR was performed whereby each DNA sample studied was amplified with four pairs of outer primers, and 1 microlitre aliquots of the PCR product were then further amplified with each of the corresponding inner primer pairs in four separate second PCR amplifications(Simmonds et al 1990). The products were analysed by agarose gel electrophoresis and the presence of DNA indicated by staining with ethidium bromide. Samples were scored as positive or negative by the presence of a DNA band of the appropriate molecular weight in the product of the second reaction. With the double PCR, negative samples do not produce visible bands. Because of the high yield of DNA from all positive samples, each reaction therefore yields a clear positive or negative result.

Tissue Typing

Blood samples were immediately added to heparin (25 units/ml blood), mixed and warmed to 37^{O}C. Carbonyl iron powder (20mg/ml blood) was added, and the mixture placed in a 37^{O}C water bath for 30 mins with occasional shaking. The sample was then placed on top of a strong magnet for at least 15 min before the upper 90% was removed. Lymphocytes were isolated by density gravity centrifugation on Ficoll/hypaque as described by Boyum (1976).

Lymphocytes were washed and resuspended in RPMI at a density of 1.5x10^{6}/ml. HLA A and B antigen was performed using a standard microcytotoxicity test (Mittal 1978). Briefly, 1microlitre of lymphocyte suspension was incubated for 30 min with 1microlitre of

each antiserum in wells of Terasaki trays, then a further 5microlitre of reconstituted rabbit serum was added as a source of complement, and incubation continued for a further 60 min. After staining with eosin and fixing with formalin, the degree of cell death was assessed using an inverted microscope.

HLA DR typing was performed using an extended (1st stage 1 hour, 2nd stage 2 hours) microcytotoxicity test after enriching for B lymphocytes by rosetting with neuraminidase treated sheep erythrocytes (Kilpatrick & Darg 1983). The antisera used were obtained partly from the United Kingdom Transplant Service and partly from commercial sources. At least 3 specific antisera were used to detect each well-defined specificity.

Respiratory virus isolation

Nose and throat swabs taken from each child were combined in a single vial of standard viral transport containing antibiotics to suppress bacterial growth and foetal calf serum to stabilise the virus. The samples were transported to the laboratory within 2 hours. If any delay was anticipated, swabs in transport medium were stored at $4^{O}C$ prior to transport. In the laboratory, cells collected on the swabs were resuspended in a small volume of transport medium prior to inoculation into primary baboon kidney, human epithelium cells (HEp2) and human fibroblasts (MRC5). The inoculated cells were incubated at $36^{O}C$ and examined twice weekly for evidence of a cytopathic effect. Further confirmatory tests were carried out on cells affected. Influenza,

parainfluenza, respiratory syncitial (RS), measles, and herpes simplex were confirmed using monoclonal or polyclonal antibodies in a fluorescent antibody test (Gardner & McQuillin 1985). Adenovirus, Coxsackie and Echo viruses isolated were identified by neutralisation tests (Hambling 1963). Rhinovirus was identified by acid stability testing. CMV was identified on the basis of characteristic cytopathic effect in MRC5 cells.

Statistical methods

The Atari K Graph programme was used for Student's t and Chi-squared test of significance for parametric data, and for Wilcoxon rank sum and Wilcoxon signed rank tests of significance for non-parametric data as appropriate. Odds ratios and confidence intervals in chapter 4 were calculated according to Algorithm AS as described by DG Thomas (Thomas 1971).

4 Limitations of the Study

The chief limiting factor on all aspects of the project was the number of women and children available to study. The study was conceived at a time when the number of known HIV seropositive women in Edinburgh was rising, and if the fertility rate among this group remained constant, it might have been expected that the number of children born to HIV seropositive women in subsequent years would increase. Thus, given that in 1985 and 1986 11 children/year were born, it might have been anticipated that in the 30 months of the study, between 40 and 50 children would be born. The numbers born every year, however, remained constant, so that only 30 were identified from birth over this period, and in 1991, the numbers have, in fact dropped. With these numbers, the confidence intervals for transmission rate would inevitably be wide. Our data have been analysed together with 9 other European centres in the European Collaborative Study of perinatal transmission of HIV. Although follow-up of children had begun in 1986, some elements, such as collection of cord blood, collection of blood from mother at delivery, tissue typing on cord blood, and routine quantification of lymphocyte subsets only began when I joined the project, so many details from the earlier pregnancies are not available.

As more women were becoming aware of their HIV status either before pregnancy, or in the early stages of pregnancy, termination would be an option. If those women opting for termination were distinct in any way from those continuing with their pregnancy, for instance, if

those women who were more clinically unwell terminated their pregnancy, this might well affect the observed rate of transmission. Although one woman who had already given birth to an infected child had a termination on developing PCP in the second trimester of a subsequent pregnancy, it has been shown that knowledge of HIV status did not affect the decision to continue with pregnancy among women at this time(Johnstone et al 1990).

Universal screening of pregnant women was not practised, and so it is possible that cases would be missed. It was hoped that good liaison with other professionals, and screening for high risk activities, with the offer of testing in the antenatal clinic would minimise this risk. In 1989, voluntary antenatal screening of all women was introduced in Edinburgh, and two mothers who may not otherwise have been identified were diagnosed as HIV seropositive during that year. Some mothers were not identified in the antenatal period, and children were enrolled when they presented with symptoms or when their mothers were later identified as being HIV seropositive. These children had to be excluded from the calculation of the vertical transmission rate.

We aimed to identify early symptoms and signs of HIV infection and to monitor the neurodevelopmental progress in children at risk of HIV infection. Because many of these children came from backgrounds of multiple deprivation, it was important to include a control group from a similar background. The follow-up included venepuncture and so it was only justifiable to perform this on babies whose mothers were at risk of seroconversion (as their sexual partner

was seropositive). The risk of maternal seroconversion was unknown, as was the risk to the child should the mother have been in the process of seroconversion in the latter part of the pregnancy. If the mother remained seronegative after one year, then the risk to the child was felt to be negligible, and most mothers refused further follow-up for research purposes alone. Although the data generated for the first year of life is useful, we were unable to address the question whether those children born to HIV seropositive women who subsequently seroreverted and were presumed uninfected were in any way distinguishable from children not at risk of HIV. Also the number of control children was smaller than the index group, partly because they were less likely to be identified, limiting the power of any significant difference that might be demonstrated. For the cord blood studies, therefore, which did not involve venepuncture of the child, samples were taken from additional seronegative women in high risk groups.

We employed the Denver developmental screen on routine evaluation of the children in the study. This was chosen because of its ease of administration, reproducibility, and suitability to be carried out at home with simple standard equipment. It is, however, a screening device, rather than intended for diagnosis, and it is possible that more subtle deficits in the functioning of the children studied may have been missed. In this, as well as the other aspects of routine assessment, observer variation was limited by the small number of personnel involved in the project.

Although the rate of vertical transmission of HIV in the

Edinburgh population was unknown, available information from preliminary studies suggested the risk to be at least 20-30% (Friedland & Klein 1987, Mok et al 1987). We anticipated, therefore being able to see a spectrum of clinical disease among at least 20 children proving to be infected. This proved not to be the case. Therefore, in order to study natural history and therapy, additional symptomatic HIV infected children were enrolled from outwith the original study population.

We were aware that numbers of symptomatic children were likely to be too small to make randomised controlled therapeutic trials impossible to perform, and that any preliminary data generated during the study on the effect of therapeutic intervention would require confirmation and further exploration with much larger groups of patients. IVIgG and AZT were used to treat symptomatic children, which was anticipated to affect natural history of the disease. Our results regarding morbidity and mortality rates might differ from earlier studies when such options were not available.

SECTION III

RESULTS

CHAPTER 1

THE VIROLOGICAL DIAGNOSIS OF VERTICALLY TRANSMITTED HIV INFECTION

Aims

The aim of this study was to compare virological methods of laboratory diagnosis in children at risk of HIV infection.

Patients and Methods

Patients

Seventy four children born to HIV seropositive women were studied. Eleven (cases 1-11) proved to be HIV infected according to the CDC paediatric classification (CDC 1987 [b]) (group 1). Sixty one were HIV antibody negative, clinically free of symptoms that suggest HIV disease and had normal test of immune function, and so were presumed uninfected (group 2); 2 were indeterminate (group 3). Thirty children born to seronegative mothers with seropositive fathers were studied (group 4), together with samples from 45 healthy HIV 1 seronegative blood donors with no risk factors for HIV infection (group 5).

Blood samples

Samples taken at routine visits were tested for HIV 1 antibody, HIV p24 antigen, and cultured for HIV as previously described in section II.3. In addition, 2 ml blood samples were collected from 10 patients in group 1, 35 in group 2, 2 in group 3, 7 in group 4 and all of group 5 into EDTA. DNA extraction and PCR was subsequently performed.

Results

All eleven children in group 1 were consistently HIV antibody positive, ten being over 18 months of age, whereas no child in group 4 was HIV antibody positive at any time. In group 2, maternal HIV antibody was lost from 6-18 months, median 12. Fig III.1.1 shows the percentage of infants positive for HIV antibody by age. The follow up of the 61 HIV antibody negative children from the time of seroreversion ranged from 3-60 months, (median 36). One child who seroreverted at 12 months had a positive result for HIV antibody by ELISA and an indeterminate Western Blot at 30 months, but has remained HIV Ab negative since. She has suffered from recurrent respiratory tract infections, and asthma, but is otherwise well. Persistent seroconversion after seroreversion has not been observed.

Tables III.1.1 and III.1.2 summarise the relevant clinical and laboratory features of group 1, further details being described in

chapters 4 and 7. Nine children have been HIV p24 antigen positive on more than one occasion during follow-up, four before the age of 18 months. In all three children followed from birth, HIV Ag was detected in serum before the onset of clinical symptoms Case 4 was positive for serum HIV Ag aged 2 and 4 months, but HIV Ag was not detected in cord blood.

Samples for HIV culture from nine children were positive on one or more occasions. HIV was isolated in culture in 3 children who were HIV Ag negative at the time, one of whom had never been HIV Ag positive. Umbilical cord blood from case 4 cultured for HIV gave a negative result, and she died before further specimens were taken. HIV has not been isolated from repeated blood samples form case 7. No sample from groups 2 or 3 was HIV Ag or HIV culture positive.

PCR was positive in all 10 cases tested, including case 7, four of whom were negative for HIV Ag at the time. No sample from groups 2 (n=41), 3 (n=2), 4 (n=7) or 5 gave PCR positive results, compared to all 22 of the samples from group 1.

All PCR positive samples identified in this study were amplified using primers which span the first and third hypervariable regions of the *env* gene (Alizon et al 1986). Polyacrylamide gel electrophoresis of the ^{35}S-labelled PCR product showed a range of length variants to be present in each sample. Each pattern of variants was specific for each patient, and distinct from that of a cloned HIV sequence. This excluded contamination by sample mixing or by recombinant DNA as a cause of

PCR positivity.

To investigate the reproducibility of this method for PCR, four aliquots of a DNA sample from each of the PCR-positive cases were amplified with the *env 1* and *env 3* primers in quadruplicate reactions. Little variability in the pattern of amplified bands were seen. Follow-up samples from each of the available infected individuals were similarly tested in order to investigate the stability of the observed patterns over time. Little variation in the pattern of bands was seen in samples collected over periods of 3-7 months.

Discussion

Our results confer closely with those of Krivine et al (1990), indicating that PCR and HIV culture have superior sensitivity to HIV Ag, although HIV Ag detection is specific. In children under 18 months, HIV Ag assay remains valuable as an early diagnostic test in assessing children born to HIV seropositive mothers as it is readily available, and requires very little serum. Nine of eleven infected children were positive for HIV Ag, 6 at a time when they were either asymptomatic or had non-specific symptoms only. Of those followed from birth, HIV antigenaemia was the first laboratory indicator of HIV infection; cases 1 and 3 also showed a raised IgG on the same sample (as defined in appendix III). HIV culture was not available at this time. Even children with severe clinical disease can be HIV Ag negative, however(Lelie et al 1988), and so a negative result is unhelpful. Sensitivity may be improved by pre-treatment to release antigen from immune complexes (Nishanian et

al 1990), but this procedure has yet to be standardised.

We observed a positive correlation between detection of HIV Ag and virus isolation. HIV isolation may be more useful diagnostically in identifying children negative for HIV Ag at the time of sampling. However, it is not normally available as a routine test and requires a much larger sample (5ml whole blood compared to 0.2ml serum for HIV Ag), which is an important consideration for babies in the first months of life. Also positive result are not easily quantified. Where both tests are available, they may provide useful complementary information.

PCR offers considerable promise for the detection of vertically transmitted HIV infection, as the method might be capable of yielding diagnostic results with high specificity and sensitivity at an age when other methods of detection may not. Although this method requires more blood than HIV Ag assay, it requires less than HIV culture, and results are available after a shorter time (48 hours rather than 3 weeks). The possibility of contamination, even with small amounts of maternal blood, leading to false positive results may limit its usefulness in diagnosis from cord blood, however. In our series, PCR did not provide a diagnosis of HIV infection in any child who did not have other virological indices of infection, as all but one child was older than 18 months at the time of testing, and case 4 was also HIV antigenaemic by 2 months of age. Further work is therefore required following up children at risk of HIV infection tested with PCR in the first months of life.

There have been three previous reports of the use of PCR in the diagnosis of vertically transmitted HIV infection, each using a single PCR amplification followed by identification of the (amplified) product with a radioactive oligonucleotide probe (Laure et al 1988, Rogers et al 1989, Edwards et al 1989). Laure et al (1988) amplified three consecutive sequences in the *gag* (1) and *pol* (2) regions. Some samples were termed PCR-positive even when positive results were obtained with only one or two of the three combinations of primers used, and such results were seen in children with proven HIV infection. A diagnosis of HIV infection based on PCR positivity was claimed in 6 of 14 newborn infants tested, but other laboratory confirmation of HIV infection was present in only one of these. Also, five out of ten children aged 2-5 years were claimed to be HIV infected on the basis of PCR positivity alone, although other laboratory confirmation in these cases was similarly lacking, and all were HIV antibody negative. Rogers et al (1989) amplified two HIV sequences in the HIV p24 and HIV gp41 regions, but had a high false negative rate, with only 6 PCR positive neonates found among 11 who later went on to develop AIDS. Edwards et al (1989) amplified two sequences in the *gag* region, but also had negative PCR results in one child with symptomatic HIV infection.

Other workers have also failed to find a clear correlation between indices of HIV infection and PCR reactivity. Imagawa et al (1989) found a number of individuals who were serum HIV antibody and HIV p24 antigen negative for several months, yet were PCR positive on more than one occasion. Conversely, the finding of extremely low

levels of provirus in certain individuals allows the possibility that false negative results may occur by testing insufficient DNA. Simmonds et al (1990) report that PBMC DNA from one seropositive individual contained only five copies of provirus per 10^6 cells. Thus considerably more than 1ug DNA would need to be amplified to ensure reproducibly positive PCR results. It is likely that patients with yet lower amounts of PBMC provirus may be identified, and these may prove refractory to screening using PCR during such stages of infection. This emphasises the importance of continued monitoring of all infants born to HIV infected mothers. In all samples in this report scoring positive or negative, there were no discrepancies between the results obtained with the four independent sets of primers from *gag, pol, env1 and env3*. The positive PCR results obtained were completely concordant with the clinical and laboratory evidence of HIV infection in these children. All control samples (groups 4 and 5) were PCR negative, and no cases of latent HIV infection were detected in groups 2 and 3.

Mullis and Faloona (1987) described the use of nested primers to increase the specificity of PCR for target sequencing. Simmonds et al (1990) have used this technique to improve the sensitivity as well as the specificity of this method for HIV detection and have reported the reliable detection of single molecules of target sequence. This is considerably more sensitive than existing methodologies. Furthermore, the higher degree of amplification possible allows the direct visualisation of amplified product, and also permits partial characterisation of HIV by virtue of its strain-dependent length variation in several regions of the *env* gene.

The demonstration of different length variants of amplified DNA is interesting. The HIV genome exhibits great diversity in nucleotide sequence, both between isolates made from different patients, and between multiple isolates made from the same patient over time (Alizon et al 1986, Starcich et al 1986, Hahn et al 1986, Saag et al 1988). In addition, certain segments of the region of *env*- encoding gp120 show extensive length variation (Alizon et al 1986, Starcich et al 1986). In a separate study of HIV infected haemophiliac patients, Simmonds et al have obtained nucleotide sequences which confirm the existence of length variants present within patients' samples. The pattern of variants is similarly patient specific (Simmonds et al 1991). Thus it has proved possible to distinguish variants of HIV both between and within infected individuals, and to distinguish genuine positive reactions from contamination by exogenous HIV sequences.

The finding of different length-variant patterns in cases 8, 9 and 10 is of particular interest, as they are siblings. The different patterns seen imply either vertical transmission of different HIV *env* variants to each child, the rapid emergence of different variants within each child (possibly due to different immune selection pressures), or both. Unfortunately, library samples of the mother's PBMC DNA spanning the pregnancies are not available for analysis. PBMC DNA samples taken from haemophiliac patients over intervals of 2 years can show considerable change in the patterns of length variants in the *env 1* and *env 3* regions in some individuals, while other individuals' patterns remain relatively unchanged (Simmonds et al 1991). PCR positivity in case 3 is

also of interest, as this patient is a dizygotic twin, the other twin being presumed uninfected (group 2) and PCR negative.

Using a double PCR to amplify hypervariable regions of the HIV genome, we have been able to diagnose vertically transmitted infection with apparently 100% specificity and 100% sensitivity, which is not the case in children under 18 months for any other test available. The distinctive HIV DNA length-variant patterns observed might be usefully exploited in further studies as a basis for the investigation of the timing of vertical transmission, by comparing the patterns amplified from serial blood samples taken from the mother throughout pregnancy with those obtained from the infant at birth and during early infancy.

TABLE III.1.1 Clinical features of HIV infected children

Case	Age*	Length of follow up	Age at 1st symptoms	Current stage	Age progressed to P2B-D	Symptoms (age)
1	69	69	6	P2A	-	Resp.infections (6) Failure to thrive (6) Diarrhoea (9) Eczema (12) Molluscum (30)
2	82(D)	64	10	Dead	-	Resp. infections (10) Diarrhoea (10) Pneumonia (12) Molluscum (42) Cardiomyopathy (69)
3	61	61	9	P2F	-	Diarrhoea (9) Resp. infections (9) Eczema (15) ITP (24) LIP (36)
4	4(D)	4	1	Dead	4	Candida (1) PCP (4)
5	46	25	-	P1B	-	Lymphadenopathy (21) Eczema (21)
6	81(D)	42	12	Dead	51	ITP (12) LIP (48) Ewing's Sarcoma(74) Encephalopathy (77)
7	76	40	33	P2A	-	Resp infections (33) Diarrhoea (33)
8	67(D)	21	24	Dead	45	Recurrent pneumonia(24) Oesophageal candida(45) Failure to thrive (36)
9	76	49	12	P2C	24	Pneumonia(12) LIP (24)
10	25(D)	14	22	Dead	24	Oral candida (20) Diarrhoea(23) Encephalopathy(24) Wasting (23)
11	43(D)	21	22	Dead	26	Recurrent pneumonia(24) Encephalopathy (25) PCP (43)

* as of 1.8.91 or at death (D)

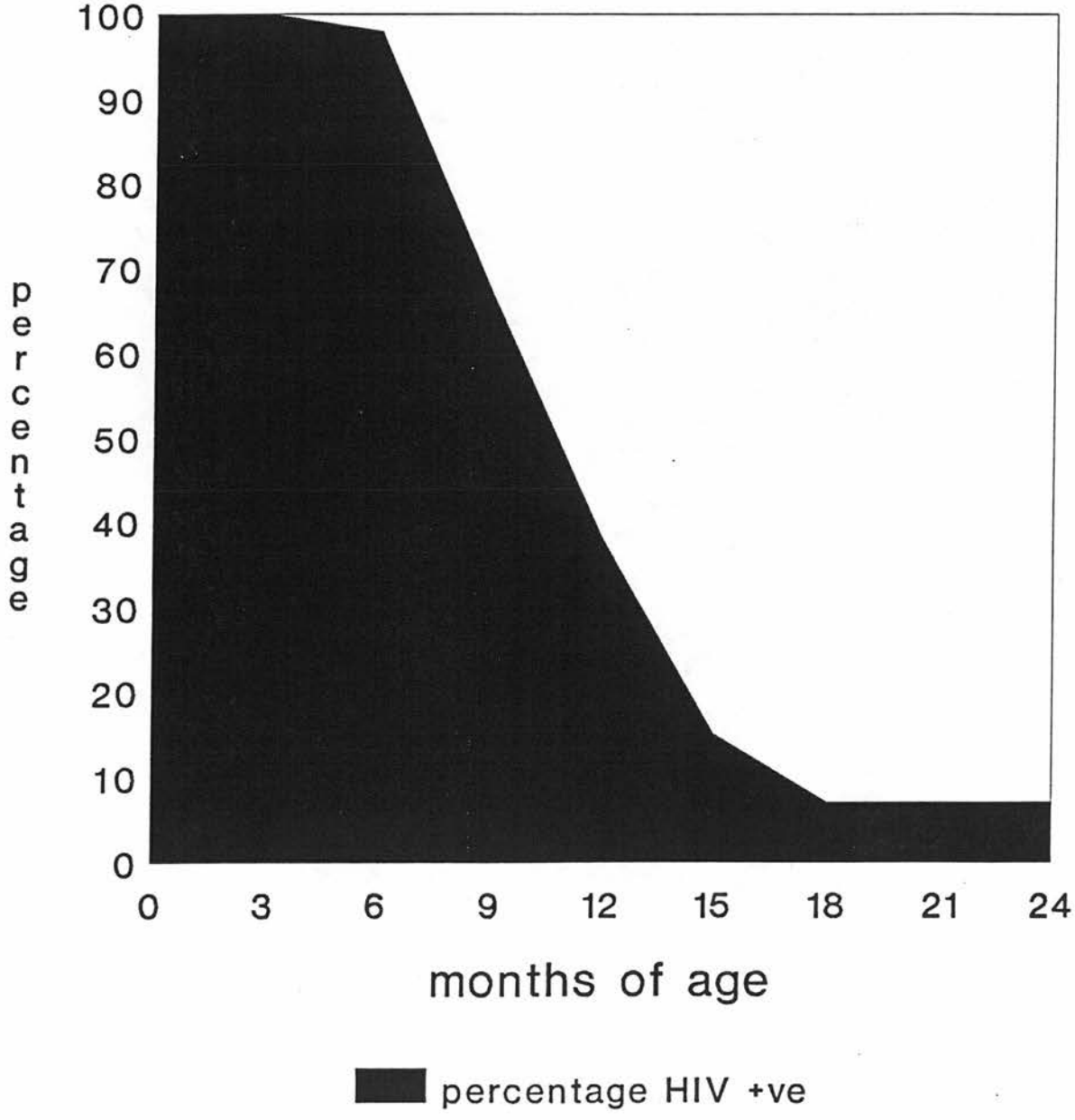
TABLE III.1.2

Laboratory data on HIV infected children

Case	1st sample available	1st HIV Ag+ (months)	Peak Ag (pg/ml)	1st p24Ab -ve (months)	Last p24Ab index	HIV culture+ (months)	HIV culture - (months)	PCR tested (months)
1	3	6	11	6	0.5	24,26,39	40	39,40,41,42
2	18	23	79	24	0	35,37,42,51	-	53,56,58
3	3	3	21	12	0.2	14,17,18	-	24,27,31
4	0	2	145	0	0	-	cord	2
5	21	21	>150	-	0.4	21,24	-	24
6	42	42	>1000	49	0	47,48,56	-	53,57,58
7	36	-	0	-	0.75	-	39,40,45	41,43.47
8	47	47	>150	47	0	57	-	66
9	24	-	0	-	0.98	33	-	42,45,47
10	12	15	>150	12	0	15	-	24
11	21	24	97	24	0	33	-	ND

FIGURE III.1.1

Percentage of infants HIV Ab positive
by age



CHAPTER 2

A PROSPECTIVE STUDY OF VERTICAL TRANSMISSION OF HIV IN EDINBURGH

Aims

To evaluate the risk of materno-foetal transmission in a group of mainly asymptomatic women, and to investigate early features of HIV infection which may identify those children at risk of perinatally acquired HIV infection who prove infected.

Subjects and Methods

From 1.1.86 to 1.2.90, 68 infants were identified and followed for a median of 44 months (range 18-70 months). The distribution of the cohort by year of birth is shown in fig III.2.1. Mothers of fifty eight infants were identified in the antenatal period and 10 were referred either subsequent to the mother's HIV antibody test being positive (9), or because of symptoms in the child suggesting HIV disease (1). These infants comprised the index group. Thirty infants were followed as controls, and cord blood taken from a further 12 controls.

Results

Maternal data

Characteristics of mothers of index and control children are

shown in table III.2.1. All were Caucasian. Mothers of 24 index infants already knew of their HIV status, whereas 34 discovered their diagnosis during the pregnancy. There were three sets of twins in the index group. Six index women had two study children, and one woman had three. There were 3 sibling pairs in the control group. Of the 57 mothers of index children, 46 were infected through IV drug use and 11 because of heterosexual contact. More index than control mothers used drugs during and after the pregnancy, but the difference between the two groups did not reach statistical significance. During the study period, one woman had PCP during pregnancy and another two subsequently died of AIDS. One index mother, and one control mother died of drug-related causes.

During the follow up period, 14 index infants and one control child were taken into foster care, with 4 subsequent adoptions in the index group. 7 index and one control were in the care of grandparents, three index children with their fathers only, and the rest were living with their mothers. Although the majority of the women had a partner some of these had unstable relationships in which the male partner was often in prison or had left home. Thirty eight of the index infants were known to have fathers who were themselves HIV seropositive.

Perinatal data

Table III.2.2 summarises perinatal data of index and control children, with the index cohort separated into children who were infected, and those presumed uninfected. The mean birth weight was

significantly lower in index children presumed uninfected (95% CI 2700g-3000g) when compared with controls, (95% CI 3065g- 3484g), and the difference remained when all children whose mothers used drugs during pregnancy were excluded from analysis. The effect of preterm delivery was allowed for by extrapolating preterm birth weight to term, along the same centile of the standard Tanner chart, and index children presumed uninfected were still significantly lighter than controls.

All but one index infant were delivered vaginally. Neonatal special care was required mainly for drug withdrawal symptoms and intrauterine growth retardation. Intensive care was necessary for 2 index infants: in one because of problems arising from preterm delivery. These consisted of meningitis with septicaemia resulting in ventriculoperitoneal shunts for hydrocephalus. The other child developed the respiratory sequelae of meconium aspiration. Multiple intracardiac tumours were discovered in one index infant, which have not caused symptoms since the neonatal period. No infant received a blood transfusion and there were no neonatal deaths. The features of HIV embryopathy (Marion et al 1986) were not observed in any infant. Although hepatosplenomegaly was documented during the neonatal period in 5 index infants, this did not persist beyond 3 months. Two index infants were breast fed (for 5 and 36 weeks each) but none from the control group.

Clinical outcome

On 1.8.91, the median age when last seen was 54 months for index infants (range 18-88 months), 18 (3-54) months for controls. No child in the index group has been lost to follow up. During the period of the study, case 4 died of PCP. Outcome of the infected children is described in detail in chapter 4, and summarised in table III.1.1. One of the children presumed uninfected has cystic fibrosis while another died aged 31 months at home from Group A Streptococcal septicaemia after a 24 hour history of diarrhoea and vomiting. Two infants died suddenly in the first three months of life, one from aspiration and the other was found dead in its cot, having been previously healthy, and so is thought to have been SIDS. Neither had any clinical or laboratory evidence of HIV infection, but their infection status remains indeterminate.

Signs and symptoms observed in index and control children are shown in table III.2.3, with index children separated into those infected and presumed uninfected. Significant lymphadenopathy was defined as the presence of nodes >0.5 cm in diameter in more than two non- contiguous sites (excluding inguinal) and persisting beyond three months. Chronic or recurrent respiratory infections were those which occurred on 3 or more occasions within a 3 month period. Recurrent diarrhoea was defined as loose stools that persisted beyond 48 hours, required treatment, and occurred on more than one occasion per month. Failure to thrive was diagnosed when the child's sequential weights declined across the centile lines.

As seen in table III.2.3, signs and symptoms were non-specific and were seen in control as well as index children. Lymphadenopathy and recurrent respiratory infections were seen in appreciably more index children, even those presumed uninfected, when compared with controls. All index children presumed uninfected had symptoms and signs which resolved with time, in contrast to those of the infected children. Neurological signs were detected only in the child with neonatal meningitis (ataxic diplegia). Five index and one control have had evidence of developmental delay on Denver screening, but the progress of the cohort as a whole has been within normal limits.

Of the 68 index children, all have received diphtheria/tetanus (DT) or diphtheria/tetanus/pertussis (DPT) immunisation, 37 inactivated polio vaccine, 31 oral polio vaccine, while measles vaccine was given to 25 children and measles/mumps rubella (MMR) vaccine to 51 children, including the symptomatic children. No adverse reactions were reported in any child or family member.

Laboratory results

Laboratory abnormalities are shown in table III.2.4. No significant differences were seen when the index children were compared with the controls. Within the index children, however, those presumed uninfected were less likely to have persistent hypergammaglobulinaemia, lymphopoenia, CD4 lymphopoenia, and thrombocytopoenia, as defined in the table. However, in the first 2

years of life, a significant difference was found only in hypergammaglobulinaemia. Abnormal laboratory results in index children presumed uninfected tended to be transient findings which returned to the normal range on repeat testing.

On sequential analysis of laboratory data by age, non- infected children and control children were indistinguishable on all parameters apart from cord blood IgG, which was significantly higher ($p<0.05$) in index children (mean 15.7g/l) than in controls (mean 11.0g/l), reflecting maternal hypergammaglobulinaemia among the HIV seropositive women. Sequential data within the index group are displayed in table III.2.5. Hypergammaglobulinaemia was noted as early as 6 months, and often predated clinical signs. The small number of samples available from infected children meant that significance was not demonstrated until 9-12 months of age. With progression of clinical disease, other useful laboratory markers were CD4 lymphopoenia and thrombocytopoenia.

Rate of maternofoetal transmission

Final analysis of the cohort was delayed until 18 months had elapsed. Five index children showed evidence of HIV disease, one of whom was referred because of symptoms, and one was first seen at 21 months after mother was found to be seropositive. Of 65 index children over the age of 18 months, 61 were HIV antibody negative, and presumed uninfected. The two who died at 3 months of age were classed as indeterminate status (CDC 1987 [b]). To calculate a transmission

rate of HIV from mother to child, the indeterminate children have been excluded. Of 66 children, 5 were HIV infected. However, it is only appropriate to include those children followed from birth in the calculation of vertical transmission rate. Three such children out of 56 were infected, giving a rate of 5.4% (95% CI 1.12 -14.87%).

Discussion

Initial reports of paediatric AIDS concentrated on children who presented with end stage disease, (Oleske et al 1983, Rubenstein et al 1983, Scott et al 1984) and they are not typical of the entire clinical range of HIV infected children. Increasing awareness and experience of children at risk of AIDS have allowed an improved classification of signs and symptoms observed in paediatric HIV infection (CDC 1987 [b]). The children in our study with proven HIV infection had common, non-specific clinical findings which might have been managed differently had they presented in another setting. The haematological and immunological abnormalities that we observed were not exclusive to the infected children, and this again underlines the importance of regular follow up with sequential data and the inclusion of control children.

Most index mothers were infected through needle sharing during intravenous drug use. As most of these women come from areas of multiple deprivation this is likely to affect their infant's health and development adversely. A previous study from Edinburgh of women who identified themselves as being at risk of HIV infection showed no significant differences in social characteristics of those who

subsequently tested positive, compared with HIV negative women (Johnstone et al 1988). Rather, similarities were seen in infant morbidity (low birth weight, intra-uterine growth retardation, preterm delivery) in both groups of women, which were increased three fold when compared with the general population. Our choice of control infants was such that both groups were comparable in social and epidemiological factors, as well as maternal intravenous drug use during pregnancy. Despite that, the mean birth weight of the index infants presumed uninfected was significantly lower than that of the controls, even allowing for maternal drug use and prematurity. This could be a direct effect of HIV during pregnancy.

More of the index children than controls were in alternative care. The chaotic lifestyles led by some parents who continue to abuse drugs were common to both groups, but in the index group, ill-health in the mothers has also meant that their children have to be taken into care. This has required close liaison with the Lothian Region Social Work Department whose staff have established guidelines for placing these children (Black & Skinner 1987).

In estimating the vertical transmission rate, we waited until children were over 18 months of age, although the recommendation was that children aged over 15 months who test antibody positive are defined as infected (CDC 1987 [b]). Our experience is that maternal antibody can persist until 18 months of age, and had we defined four such children as infected, we would have overestimated the rate of transmission. Children referred because of HIV related symptoms

would also add to an over-estimate of infection. Therefore in the calculation of infection risk, the more accurate estimate of risk will be derived from the inclusion only of those children monitored from birth. The risk of vertical transmission from our data was 5.4%, lower than that previously reported (Mok et al 1987, ECS 1991, Minkoff et al 1987, Blanche et al 1989), although still within the confidence intervals of the European Collaborative Study (ECS 1991). Our results must be interpreted with caution, however, as numbers are small, and as yet, the prognosis for children aged over 18 months who lose maternal antibody is unknown. Although not observed in our cohort, children have been reported to lose antibody only to seroconvert later on in childhood (Aiutu et al 1987) while others have been documented to remain antibody negative with positive antigen tests and virus culture (Mok et al 1987, ECS 1988, Borkowsky et al 1987).

The virological diagnosis of HIV infection has been discussed in the previous chapter. We were unable to detect any other laboratory parameter which consistently predicts HIV disease when sequential index and control data were compared. In HIV infected children, immunoglobulin concentrations (especially IgG) started to rise before clinical signs were obvious. Early clinical manifestations were usually non-specific, although one child presented with oral and cutaneous candidiasis rapidly followed by PCP, which is a well recognised pattern (ECS 1991). When recurrent respiratory infections, lymphadenopathy and hepatosplenomegaly are seen in conjunction with laboratory abnormalities (hypergammaglobulinaemia, CD4 lymphopoenia, thrombocytopoenia), a diagnosis of HIV infection can be strongly

suspected in a child especially when a mother has engaged in high risk activities. Our study therefore stresses the importance of continued careful follow up of all children at risk of HIV infection, to detect early markers of infection as well as to determine the outcome of those who appear symptom free.

TABLE III.2.1

Characteristics of mothers of 68 index and 30 control children

	Index n=68	Control n=30	
	n (%)	n (%)	p
Mean age at delivery	24	23.4	NS
Single mother	23 (34)	8 (27)	NS
First born	30 (43)	17 (59)	NS
Drug use during pregnancy	27 (39)	5 (17)	NS
Drug use since pregnancy	29 (42)	6 (21)	NS
Infant in alternative care	22 (32)	2 (7)	<0.05^

TABLE III.2.2

Perinatal data of 66 index and 30 control children

	Index		Control
	Infected	Uninfected	
	n=5	n=61	n=30
Boys	0	27	17
Born <37 weeks' gestation	0	11	3
Birth weight <10th centile	1	19	3
Neonatal special care	1	16	4
Mean birth weight (g)	2925	2860*	3285*
Mean birth weight (g) of babies born to drug free mothers	2925(n=4)	3028~(n=37)	3394 (n=25) ~

* p<0.01}

~ p<0.02} Student's t test

^ Chi-squared test

TABLE III.2.3

Clinical signs and symptoms in 55 index and 30 control children in the first 18 months of life.

	Index		Control
	Infected n=5	Uninfected n=50	n=30
Recurrent resp. infections	4 (80%)*	12 (24%)*	2 (7%)
Recurrent diarrhoea	3 (60%)	10 (20%)	4 (13%)
Eczematous rash	4(80%)˜	9 (18%)˜	1 (3%)
Sig. lymphadenopathy	4 (80%)˜	9 (18%)˜	2 (7%)
Hepatosplenomegaly	4 (80%)˜	11 (22%)˜	2 (7%)
Failure to thrive	1 (20%)	3 (6%)	0
Oral candida	1 (29%)	2 (4%)	3 (10%)
Cutaneous candida	3 (60%)	19 (38%)	4 (13%)

TABLE III.2.4

Laboratory abnormalities in 61 index and 30 control children in the first 2 years of life.

	Index		Control
	Infected n=5	Uninfected n=56	n=30
Hypogammaglobulinaemia ˆ	0	18 (32%)	6 (20%)
Hypergammaglobulinaemia ˆ	4 (80%)#	4 (75)#	1 (3%)
Neutropoenia (<1.0x10^9/l)	1 20%)	3 (5%)	3 (10%)
Lymphopoenia (<2.8x10^9/l)	1 (20%)	2 (4%)	2 (7%)
CD4 lymphopoenia (<1.0x10^9/l)	1 (20%)	1 (2%)	2 (7%)
CD4/CD8 <1	2 (40%)	5 (9%)	1 (3%)
Thrombocytopoenia (<100x10^9/l)	2 (40%)	3 (5%)	0

* p<0.05 }
˜ p<0.01 } Chi-squared test
# p<0.001}

ˆ Normal ranges for IgG are found in appendix III

TABLE III.2.5

Sequential laboratory results index children

	Cord		3 months		6 months		9-12 months		15-18 months		21-24 months	
	I	**NI**	**I**	**NI**	**I**	**NI**	**I**	**NI**	**I**	**NI**	**I**	**NI**
n	1	22	3	49	2	42	2	54	3	57	4	35
IgG (g/l)	10.2	15.7	5.0	4.5	10.2	4.2	12.4	6.0*	14.9	7.5*	16.6	7.7˜
IgA (g/l)	0.1	0.42	0.5	0.6	1.4	0.5	1.3	0.6	1.5	0.7	1.3	0.7
IgM (g/l)	0.2	0.29	1.0	1.26	1.3	0.9	1.0	1.0	1.2	1.2	1.5	1.1
CD4 lymphocytes($x10^9$/l)	2.34	1.75	1.9	3.35	ND	3.4	ND	2.75	1.32	2.39	1.28	2.03
Platelets ($x10^9$/l)	323	277	390	485	281	400	249	352	180	372	115	380*

* $p<0.05$}

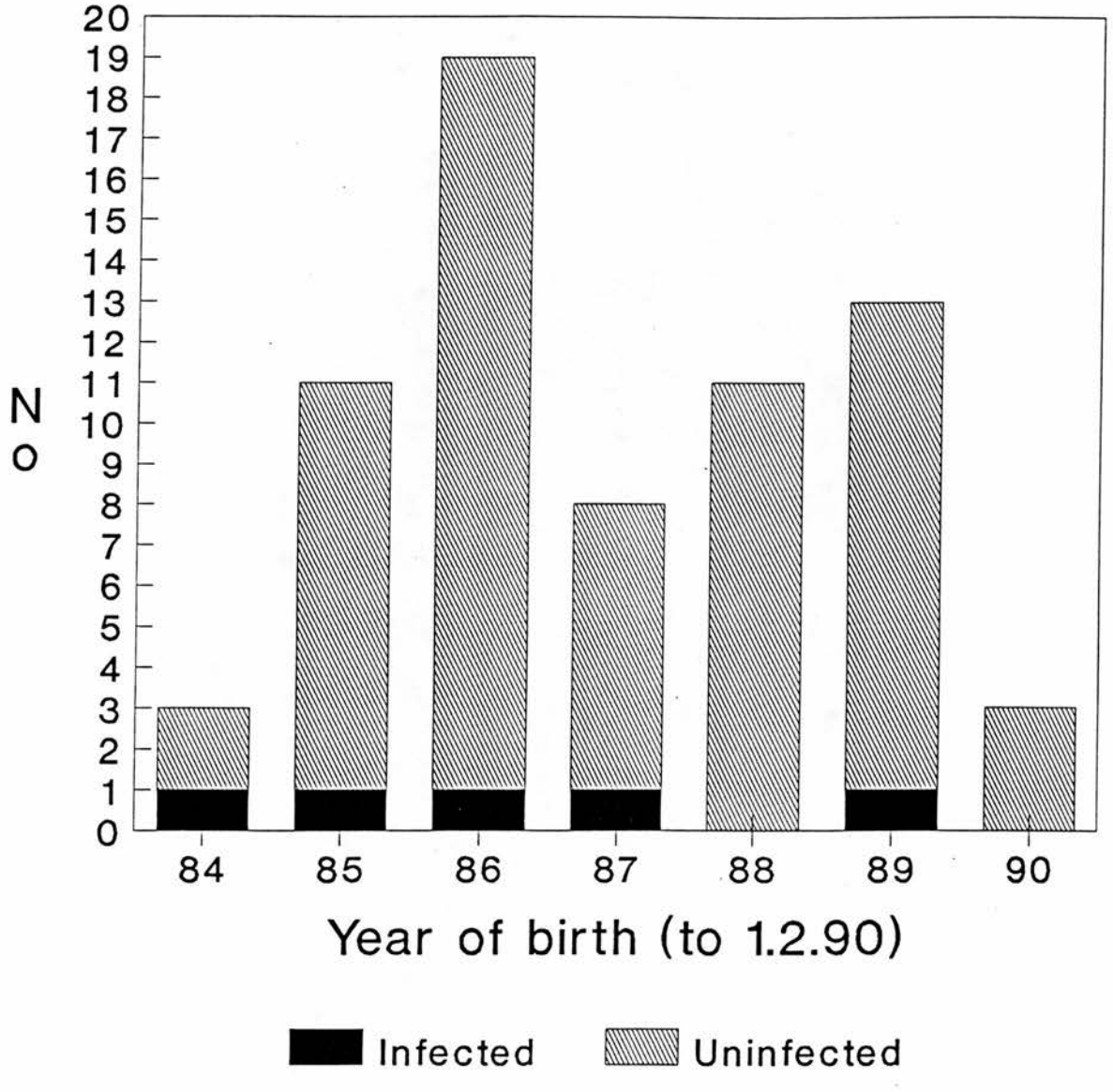
˜ $p<0.02$ } Student's un-paired t test

I Infected

NI Not infected

FIGURE III.2.1

Distribution of index children
by year of birth



CHAPTER 3

DO MATERNAL FACTORS INFLUENCE THE RISK OF VERTICAL TRANSMISSION OF HIV?

Aims

Having estimated the current rate of vertical transmission in Edinburgh at 5.4% which is lower than all published series, for reasons which are unclear, we analysed some factors which might influence HIV transmission from women to their offspring over 7 years in order to identify those which may have contributed to the low vertical transmission rate.

Subjects, Materials and Methods

Mothers and infants including cases 6-11, were followed as previously described. Data from fifty six children and their mothers (including case 12, who was identified after the conclusion of my research fellowship) followed prospectively, and fourteen children diagnosed after the neonatal period were analysed.

Results

Seventy children of 58 HIV seropositive mothers (3 pairs of

twins, 7 sibling pairs, and 1 sibship of 3) were followed for a median of 4 years (range 0.5-7 years) post-delivery. Twelve children (cases 1-12) born to 10 women were infected. Their case histories are outlined in Appendix II. One woman had 3 infected children (cases 8, 9, 10). The mothers of cases 4 and 12, who both developed AIDS during pregnancy, had each had a previous uninfected child 4 and 7 years before the birth of the index case, when they were seropositive but asymptomatic. The mothers of cases 5 and 7 went on to have an uninfected child 2 years later.

Maternal characteristics

These are summarised in table III.3.1. Forty four (75%) of women were infected through needle sharing, 13 were infected heterosexually, and one is thought to have received an infected blood transfusion. As illustrated in figure III.3.1, there was no difference in the mean duration of mother's infection between those who transmitted and those who did not. However, five of the nine pregnancies within a year of seroconversion resulted in infected children compared with 7 of the 61 in subsequent years ($p<0.001$). It would appear that a smaller proportion of women whose risk factor was intravenous drug use transmitted to their children. However, more of the mothers infected heterosexually had pregnancies within the first year of seroconversion. Excluding these 9 pregnancies, 4/7 mothers who transmitted, compared with 47/54 who did not had used intravenous drugs; this difference is not significant (OR 3.91, 95% CI 0.48-27.1).

Table III.3.2 contains details of pregnancy outcome. There was no difference between the 2 groups on any parameter measured. The Caesarean section rate was very low in our series. The diagnosis of chorioamnionitis based on maternal pyrexia during labour and macroscopic placental appearance was not made in either group, although the mother of one infected child (case 7) had an unexplained pyrexia 48 hours after delivery. The only placental abnormalities seen were infarction in 2 mothers of uninfected children. The mother of an uninfected child who later died of AIDS suffered a severe wound infection following her Caesarean section. The mother of cases 8-10 breast fed, but all the other infected children were artificially fed.

At the start of their pregnancies, 56 women were asymptomatic. Two women were classified as stage 4.C2, one on the basis of oral hairy leukoplakia, and one suffered oral candidiasis. Neither produced infected children. Both women who developed PCP as their first AIDS defining illness during pregnancy bore HIV infected children. Three mothers of HIV infected children and 2 mothers of 3 uninfected children have died of AIDS. Outcome for the mothers of HIV infected children is summarised in table III.3.3.

During the period of follow-up, nine mothers of infected children have progressed to stage IV at a median of 3 years (range 0-6.5), and 19 mothers of uninfected children at a median of 5 years (range 1-7). As shown in figure III.3.2, women who bore infected children were likely to progress more rapidly to stage IV during the period of follow up (log rank statistic 4.603, giving a p value of 0.032).

Laboratory data are shown in table III.3.4. Mothers of all three infected children tested during pregnancy had CD4 counts less than $300x10^6/l$. Counts are available for the majority of women within 1 year of delivery, when a greater proportion of women bearing infected children had low numbers. Maternal HIV antigen and p24Ab were not discriminatory. In nineteen women, PBMCs were cultured for HIV during pregnancy. HIV was isolated in 4. None of the 19 transmitted to their children, and so no conclusion can be drawn.

Discussion

The number of pregnancies resulting in HIV infected children among this group was small, and there may be associations with increased risk of transmission which failed to reach significance in our study. However, important associations have been identified. In Edinburgh we have detailed seroconversion data, enabling us to demonstrate the greater risk of transmission of HIV to a child born shortly after mother's seroconversion. During primary HIV infection, high titres of cytopathic virus have been demonstrated in plasma (Clark et al 1991), and so the foetus may be more susceptible to infection on exposure to this high virus load, especially before any maternal immune response has been mounted. In addition, although few mothers were symptomatic at delivery, but the only two with AIDS both transmitted to their children, supporting previous studies suggesting that advanced immunodeficiency increases the risk of vertical transmission (Ryder et al 1989). Similar variations in a woman's infectivity

have been suggested from heterosexual transmission studies (May 1988). Counselling of HIV seropositive women wishing to have children should take these factors into account.

Because of the small number of children born by Caesarean section, we were unable to explore any relationship between the mode of delivery and risk of transmission. In a retrospective twin study, Goedert et al (1991) have suggested that contact with the cervix and birth canal may be important, but others have failed to demonstrate any association (Semprini et al 1987, Lindgren et al 1991). It has been suggested that breast feeding carries a greater risk of transmission than bottle feeding (Blanche et al 1989), and the recent European study indicates and odds ratio of 2.25 (95% CI 0.97-5.23) in favour of transmission for breast fed infants compared with those exclusively bottle fed (ECS 1992). Studies of post-natal transmission of HIV indicate that breast milk may be an efficient route from recently infected mothers (Ziegler et al 1985, Van de Perre et al 1991). Only 6 infants in our study were breast fed, and although it is interesting that the mother of cases 8-10, who transmitted twice at times of 'low risk', breast fed on all three occasions no conclusions can be drawn. Current guidelines are to discourage HIV seropositive women in the UK from breast feeding (Royal College of Obstetrics and Gynaecology 1987). For the majority, however, the decision to bottle feed had been made before any discussion of the possible risks of breast feeding had occurred. Only one woman in our study who breast fed was aware of her HIV status (and the guidelines) at the time.

Our data also suggest that asymptomatic women who bear HIV

infected children may progress more rapidly to stage IV than those who do not. A previous study has suggested that the progression of disease in this group of women is related to the duration of maternal HIV infection (Lindgren et al 1991). Our knowledge of seroconversion times has meant that in a larger study population we have demonstrated no difference in the duration of maternal HIV infection between the two groups which would account for this; rather the data suggest a difference in rate of progression. If bearing an infected child is confirmed as being a bad prognostic indicator, this may affect decisions regarding the introduction of anti-retroviral therapy for the mother.

Although the majority of infected children were born before CD4 counts were measured routinely, the data we have supports an association between low CD4 counts and transmission, as was found in Zaire (Ryder et al 1989). Other laboratory markers of HIV disease progression include HIV antigenaemia and loss of p24Ab (Forster et al 1989). In common with other groups (Goedert et al 1989), we found no correlation with p24Ab levels. The presence of HIV Ag in maternal serum has been associated with transmission by some groups (Scarlatti et al 1991) but not by others (Devash et al 1990). In our study, a greater proportion of women who transmitted HIV to their children were HIV antigenaemic, but the difference did not reach statistical significance. Further studies on larger groups of women and children are therefore required.

In our prospective study, the rate of perinatal transmission of HIV in Edinburgh is 5.4% (95% CI 1.12 14.87%), lower than the

published series, although still within the confidence interval of the European Collaborative Study (ECS 1991) and indeed, the rate among asymptomatic mothers followed prospectively from birth for at least 18 months is 2/51 (3.9%, 95% CI 0.48-13.46%). At present, few of our mothers fall into the higher risk categories identified in this study. In the coming years, a greater proportion of HIV infected women in Edinburgh will have more advanced disease. Future studies may test the hypothesis that the perinatal transmission rate will increase with progression of HIV disease in the mothers.

TABLE III.3.1

Maternal characteristics

	Infant		
	Infected	**Uninfected**	**p**
Maternal age (mean)	20.9	24.4	NS
Previous pregnancies	1.5	1.43	NS
Previous live births	1.16	1.01	NS
History of drug use	4 (33%)	49 (84%)	<0.001
Drug use during pregnancy	2 (17%)	21/54 (39%)	NS
Partner HIV +ve	12 (100%)	38/48 (79%)	NS

TABLE III.3.2

Outcome of pregnancy

	Infant		
	Infected n=12	**Uninfected n=58**	**p**
Preterm <37 weeks	1 (8%)	9 (16%)	NS
Birth weight (mean)	2735g	2850g	NS
Birth weight <10th Centile	2 (17%)	13 (22%)	NS
Caesarean section	0	3 (5%)	NS
Placental abnormalities	0	2 (3%)	NS
Breast feeding	3 (25%)	3 (5%)	NS

TABLE III.3.4

Laboratory characteristics of the mothers

	Infant				
	Infected	**Uninfected**	**p**	**OR**	**CI**
CD4 count at delivery $<300x10^6/l$	3/3(100%)	8/49(16%)	<0.01	0	0. 0.58
1st available CD4 count $<300x10^6/l$	7/12(58%)	7/49(14%)	<0.01	8.4	1.7, 43
Maternal HIV Ag +ve during pregnancy	2/9 (22%)	2/38(5%)	NS	5.14	0.3, 78
Maternal HIV Ag +ve at any time	5/12 (42%)	8/56 (14%)	NS	4.3	0.8, 20
Maternal p24Ab -ve at any time	4/10 (40%)	14/41 (34%)	NS	1.3	0.2, 6.5

TABLE III.3.3

Clinical features of mothers of HIV infected children

Case no.	Seroconversion date	Delivery date	CDC stage at delivery	CD4 count at delivery	HIV Ag	Year diagnosed CDC IV	Outcome mother	Outcome child
1	7/83	8.11.85	II	1.32	-	-	Stage III	Stage 2A on AZT
2	1/84	9.11.84	II	0.82	-	1988	Dead 1989	Dead 1991
3	4/84	23.8.86	II	0.27	-	1989	4.32	P2F on IVIgG
4	6/83	12.1.89	IV	0.04	+	1988	Dead 1991	Dead 1989
5	9/87	14.12.87	II	0.34	+	-	Stage III	P21B
6	9/83	29.7.84	II	ND	ND	1990	4.32	Dead 1991
7	6/84	14.5.85	II	ND	ND	-	Stage II	P2A
8	1/83	1.6.83	II	ND	ND	1990	4.35	Dead 1989
9	1/83	3.6.85	II	ND	ND	1990	4.35	P2C on IVIgG
10	1/83	8.12.86	III	0.17	-	1990	4.35	Dead 1988
11	2/83	1.3.85	II	ND	ND	1987	Dead 1987	Dead 1988
12	1/84	14.9.90	IV	0.16	-	1990	4.31 on AZT	P2B

FIGURE III.3.1

Distribution of deliveries
by time since maternal seroconversion

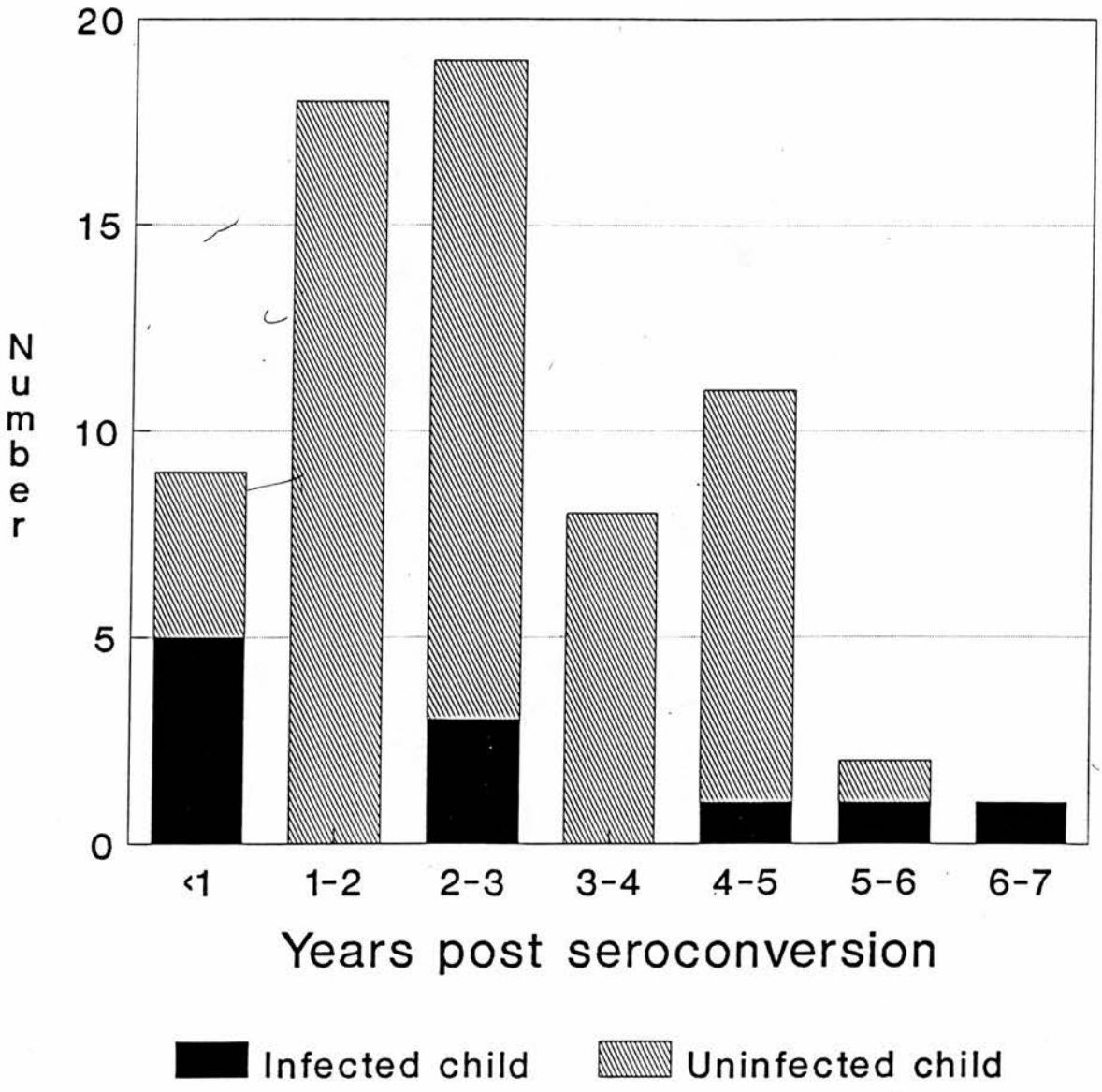
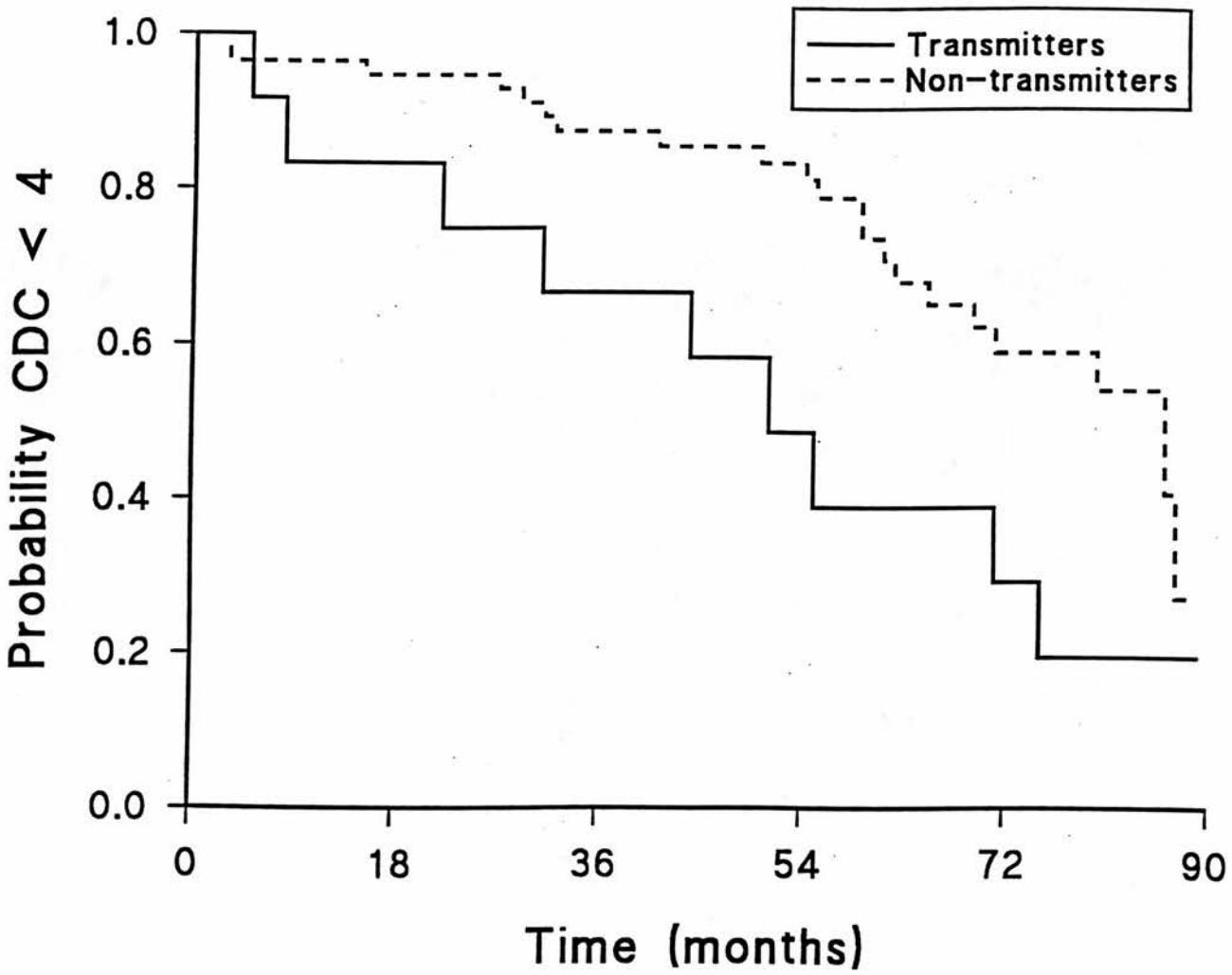


FIGURE III.3.2

Kaplan Meier plot of progression to CDC stage IV in women following pregnancy



CHAPTER 4

THE SPECTRUM OF CLINICAL DISEASE IN HIV INFECTED CHILDREN

Aims

To describe the natural history and clinical manifestations of HIV infection in the children studied, with reference to the current literature.

Methods

All 11 children born to HIV seropositive women identified during the study are described, together with the clinical features of case 13, infected through transfusion.

Results

The 11 perinatally infected children were identified at a median age of 21 (range 0-46) months and followed for a median of 33 months (range 4-66). Full details of their case histories are found in appendix II.

Table III.4.1 outlines the progress of the group. All were asymptomatic at birth, with a mean birth weight of 2.77kg. Earliest symptoms and signs were seen in case 4, with persistent candidal infection after 1 month, but 6 (55%) had developed symptoms before

the age of 1 year (95%CI 23.38-83.25%), and a further 3 had persistent lymphadenopathy. By the age of 2 years, 9/11 (82%) were symptomatic (95% CI 48.22-97.72%). Early non-specific symptoms and signs are detailed in table III.4.2.

During the period of follow up, 7 perinatally infected children have progressed to AIDS as defined in table 3, appendix I, the median age of progression being 45 months. One child remains asymptomatic at 42 months. Six children have died at a median age of 43 months, range 4-82 months. In both the two surviving children with AIDS, the indicator disease was LIP.

Features of advanced HIV disease in cases 1-11 are listed in table III.4.3. One child died in the first year of life (mortality 9%, 95% CI), 3 in the first 5 years of life (mortality 27%, 95% CI). Six (55%) died at a median of 55 months, range 4 - 82 months. Case 13 has also progressed to AIDS, but being infected through a blood transfusion is excluded from consideration of natural history of vertically acquired HIV infection: he is included in the subsequent account of clinical features of HIV infection in children. PCP was the terminal illness in 2 children, and in no other child was the diagnosis of PCP proven. No other opportunistic infections were documented. Three children with signs of encephalopathy died within 18 months of its development. Other terminal illnesses were bacterial pneumonia (case 8), HIV wasting syndrome (case 10), and cardiac failure secondary to cardiomyopathy (case 2).

In contrast, a more favourable outcome has been noted in those children who have developed radiological evidence of LIP. In all four, the diagnosis was made during an acute respiratory infection, when chest X rays showed typical changes which did not resolve on follow up films. Three remained free of chronic respiratory symptoms but case 13 had reduced exercise tolerance and finger clubbing and has required steroid therapy.

Of those infected children who have not progressed to AIDS, case 5 remains asymptomatic at the age of 46 months. Cases 1 and 7 have had symptoms in the past, but are currently well at 69 and 76 months.

Discussion

Our cases illustrate well the spectrum of HIV disease in children with perinatal infection described in larger studies (Scott et al 1989, ECS 1991). The children we studied were normal at birth, but congenital HIV infection may give rise to low birth weight, microcephaly and hepatosplenomegaly, and some have described a dysmorphic syndrome (Marion et al 1986). Most infants however, present later as did our children, with non-specific signs, such as recurrent respiratory infection, eczema, diarrhoea and failure to thrive (ECS 1991). In only one child was AIDS diagnosed in the first year of life. This contrasts with the early data from the USA, where in 50% children with AIDS, the diagnosis was made during the first year of life, and in 82% by 3 years of age (CDC 1986 [a]). Larger prospective studies have documented the

mortality from HIV in the first year of life of 17%, 26% having developed AIDS by one year(ECS 1991). Those surviving the first year, however, show a tendency to improve rather than deteriorate during the second year.

Bacterial Infection

Abnormal humoral immunity is a prominent feature of HIV infection in children, and recurrent or serious bacterial infections occur more commonly in paediatric HIV infection than in adults (Rubinstein 1986). Serious bacterial infection in the children in our study were limited to the respiratory system. We were unable to isolate the causative organisms in all episodes of pneumonia, but the commonest organisms reported are Haemophilus influenzae, Pneumococcus, and Salmonella (Bernstein, Krieger et al 1985), so our therapy reflected this knowledge. Our patients developed their infections while at home, but in hospitalised patients, nosocomial pathogens may present problems, and a knowledge of the locally prevalent organisms influence the choice of antibiotics used. In addition to pneumonia, we have also seen chronic bacterial infections such as otitis media and sinusitis, and also skin and soft tissue infections, and urinary tract infections as reported by other groups(Bernstein et al 1985 [a], Shannon & Ammann 1985).

Viral Infection

Children who acquire HIV perinatally, may have an immune system incapable of developing effective antibodies to the common

viral infections of childhood. They are especially at risk of severe complications of viral infections. The role of virus infections in the respiratory tract is discussed in chapter 5. Two infants born to HIV seropositive mothers who proved uninfected, and one of the HIV infected children (case 1) developed measles, which in all cases was uncomplicated. In the immunocompromised child measles may take an atypical course and fatal measles pneumonia has been described in the absence of any identifiable rash (Markowitz et al 1988). Measles may occur despite previous immunisation (CDC 1988 [a], Krasinski & Borkowsky 1989). Although 4 children reported contact with chicken-pox, none developed clinical infection, which may become disseminated with pneumonia and encephalitis, and chronic cutaneous varicella zoster can occur. Antiviral treatment has become available with the advent of acyclovir. However, treatment may fail if pneumonitis has already developed, making early recognition and prompt administration essential (Nyerges et al 1988). There is concern that as the number of people receiving treatment for varicella zoster increases, resistant strains will appear. This has already been demonstrated in a child with AIDS who developed chronic cutaneous lesions despite multiple courses of acyclovir (Pahwa et al 1988). In children receiving regular intravenous immunoglobulin therapy, some passive protection may be afforded, assuming the donors are immune. In the cases of reported exposure, hyper-immune measles and zoster immunoglobulin were given but prophylaxis must be given early to be of use (Krasinski & Borkowsky 1989).

CMV is commonly isolated from HIV infected children, but its role in symptomatology can be difficult to ascertain(Jacobson & Mills 1988).

This virus was isolated from the respiratory or urinary tracts in four children in our study (cases 1,2,3,7), and all remained well with no evidence of disseminated infection. Primary herpes family infections may present only with a spiking pyrexia which resolves after a few days (Pahwa et al 1988). Dissemination of live virus can lead to severe complications involving many organ systems. Once established, these infections are very difficult to treat. Early diagnosis of these infections is therefore very important. For symptomatic disease, gancyclovir (DHPG) and foscarnet have had reported efficacy in adults (Morris 1988 [a]), but to date, little experience has been reported in children.

HIV infected children may have atypical antibody responses to Epstein Barr virus (Birx & Redfield 1986). EBV genome has been found in lymph nodes, lung and salivary glands of these children (Andiman et al 1985), and has been implicated in the pathogenesis of LIP, polyclonal polymorphic B cell proliferative disorder, and lymphomas (Andiman et al 1985, Fackler et al 1985). No such work was carried out on our children.

Fungal Infection

Candidal infection of the mouth and napkin area is common in all children in the first year of life, and usually responds to nystatin or miconazole. The European Collaborative study indicates that persistent oral candidiasis is highly predictive of progression to AIDS(ECS 1991). In case 4, who developed PCP at 4 months, persistent mucocutaneous candidiasis was the first clinical sign of immunodeficiency. Six other children (including case 13) have had oral

candida, extending into the oesophagus in case 8. Case 8 was treated with ketoconazole but clotrimazole has also been shown to be effective (Young 1987). Fluconazole is reported to be more effective than ketoconazole in adults but was not yet licensed for use in children (de Wit et al 1989). In extensive oesophageal candida, amphoteracin B and flucytosine intravenously may be required, and the condition is likely to recur unless maintenance treatment is initiated(Glatt et al 1988). We did not see other fungal infections, such as cryptococcosis which are much less common in children than adults (Falloon et al 1989).

Opportunistic Infection

Pneumocystis carinii pneumonia was the only opportunistic infection diagnosed in our group, and is discussed in detail in chapter 6. Infection with latent organisms such as toxoplasma are less common than in adults(Pizzo et al 1988 [a]). Other important opportunistic infections which have been described include disseminated mycobacterium avium-intracellulare infection and cryptosporidiosis (Rogers et al 1987, Selik et al 1987). In both these infections, treatment has proven to be difficult. In the former, clofazimine plus rifampicin and ethambutol may be tried (Young 1987), but in cryptosporidiosis, the role of spiramycin has yet to be established (Current et al 1983, Soave & Johnson 1988).

Respiratory Disease

Four children (cases 3, 6, 9, 13) developed LIP. This is an AIDS-defining illness found almost exclusively in children, and occurs in

about half of the children with AIDS (Rogers et al 1987). It is characterised by the progressive development of diffuse bilateral reticulonodular infiltrates, sometimes with hilar and mediastinal lymphadenopathy (Rubinstein et al 1986). Case 13 was symptomatic, having decreased exercise tolerance, with tachypnoea, and digital clubbing. Case 9 had a chronic cough, and was prone to intercurrent chest infections but was otherwise asymptomatic. All four children had generalised lymphadenopathy, but glands were particularly enlarged in cases 9 and 13, who also had hepatosplenomegaly, and very high levels of IgG (>50g/l). Case 6 had intermittent salivary gland enlargement. These features are all known to be associated with LIP. The definitive diagnosis is made by open lung biopsy, but in our series a presumptive diagnosis was based on the typical clinical and radiological features. Although leading to chronic hypoxaemia, and susceptibility to superimposed acute respiratory infections, LIP is associated with a relatively good prognosis, with a median survival of 72 months (Rubinstein et al 1986, Scott et al 1989, Blanche et al 1990). Three of the four children are still alive, at a median age of 76 months, and case 6 died at 81 months of causes unrelated to the respiratory system. Elevated titres to EBV have been described in children with LIP (Birx & Redfield 1986), but this was not a feature observed in our series. HIV has been cultured from bronchial lavage fluid (Ziza et al 1985); this procedure was not carried out in the children we studied. Case 13 was given corticosteroids with symptomatic improvement, although this is potentially hazardous in an already immunocompromised host (Rubinstein 1986). Management may also include supportive measures, such as continuous oxygen therapy. Zidovudine has not been shown to affect

its course(Helbert et al 1987), but in some children, spontaneous resolution may occur.

Neurological Disease

The central nervous system is susceptible to the consequences of immune compromise in terms of meningitides and encephalitides, and also to cerebrovascular disease, and malignancies, in particular, primary CNS lymphoma (Epstein et al 1988 [a]). Of the four children in the study who had CNS abnormalities, three were investigated by CT scan and 2 by MRI, but no focal lesions were demonstrated. Like the majority of children with neurological symptoms (Belman et al 1988), they were felt to be suffering from a primary HIV encephalopathy. We have observed many of the common manifestations of HIV encephalopathy, which include acquired microcephaly, cognitive changes, delay in, or loss of motor milestones, and bilateral pyramidal tract signs. In case 11, encephalopathy presented early in the clinical course and case 13 had gross motor symptoms intermittently. In cases 6 and 10, CNS manifestations occurred late in the terminal phase of the disease.

More subtle neurodevelopmental abnormalities have been reported to occur in most infected children (Pizzo et al 1988 [a]). Even in those who have no symptoms, sequential assessment can demonstrate a decline in IQ scores over time in children who may be assessed as being in the normal range on an isolated occasion. However, data may be difficult to interpret in series where there has been no control group of children of the same socio-economic

background, with equivalent patterns of maternal drug use. Developmental delay in these children is likely to be multi-factorial when compared to a 'normal' population, and we have found no difference between cases 1-5, 7-9 and uninfected index children. Zidovudine has been shown to result in a significant improvement in neurological function(Pizzo et al 1988 [b], Blanche et al 1988). This was certainly so in case 11, and probably so in case 13. However, despite these encouraging findings, zidovudine may only arrest or delay further deterioration, and case 11 eventually deteriorated neurologically despite being on zidovudine.

Nutrition

Seven children failed to thrive during the period of study. Failure to thrive is a common feature of chronic illness in childhood, and of children in the socio-economic group to which many HIV seropositive families belong, even in the absence of identifiable disease. Thus good dietary advice is important even for those children who are asymptomatic, or who have only early signs of HIV infection. Attention to nutrition is particularly essential for children whose energy requirement is increased by chronic infection. Persuading an ill child to eat is sometimes impossible. Nasogastric or nasoduodenal feeding, continuously or overnight, becomes a possible method of treatment, and was instituted in case 8 for some time. In no child was parenteral feeding contemplated, not only because of the hazards of indwelling catheters in an immunocompromised child, but also because in those

children unable to tolerate enteral feeding their general condition was such that it was felt not justified to prolong life in this manner.

In HIV infection, nutritional problems may be compounded by diarrhoea with or without vomiting, and recurrent or chronic diarrhoea was an early feature of symptomatic disease in eight children. Commonly no organism is isolated, but in case 10, whose severe diarrhoea and wasting was the cause of her death, an enteropathogenic E Coli was grown. Other organisms implicated include rotavirus, CMV, cryptosporidium, Isospora belli, and atypical mycobacteria(Young 1987). For those in whom no pathogen can be isolated, the cause may be multifactorial, as discussed in section I.2. A regimen of oral gentamicin and cholestyramine has been suggested to treat those children whose diarrhoea persists despite discontinuing oral feeding and giving intravenous fluid replacement(Shapiro & Kain 1988). Zidovudine has been demonstrated to be effective in some cases(Blanche et al 1988), and was commenced for this reason in case 10. It may be that in this case, however, treatment was started too late to be of any benefit.

HIV associated Thrombocytopoenia

Three children developed thrombocytopoenia (platelet count $<20x10^9/l$), including case 13. ITP was the presenting symptom of HIV infection in cases 6 and 13. HIV related thrombocytopoenia is indistinguishable clinically from idiopathic immune thrombocytopoenia and commonly presents long before any symptoms or signs of

immune deficiency develop (Saulsbury et al 1986). The exact mechanism remains unclear, but patients with HIV-related ITP have markedly elevated platelet IgG, IgM and C3C4, as well as serum immune complexes containing anti-HIV 1 antibody (Karpatkin et al 1988). Most children remain asymptomatic, requiring no intervention. However, spontaneous bleeding and bruising occurred in all three in our series on occasions when platelet counts dropped to below $20x10^9/l$. Case 13 was treated with high dose immunoglobulin and his platelet count reverted to normal within 6 months. Case 6 continued to have spontaneous nosebleeds and petechiae while receiving regular IVIgG at 200mg/kg 3 weekly, but never developed more serious bleeding problems. His platelet count returned to normal at the time when he lost p24Ab, and only dropped again when his marrow was depressed during chemotherapy for his sarcoma.

Case 3 developed ITP while receiving IVIgG, and her platelet count dropped persistently to below $5x10^9/l$. High dose intravenous immunoglobulin (1g/kg), shown to be effective in some cases even when refractory to steroid therapy (Pollack et al 1988), led to a transient rise in platelet count, but this was not sustained. Following favourable reports of its use (Oskenhendler et al 1988) anti-D has been used, which is maintaining her platelet count above $20x10^9/l$. No child has been given corticosteroids, and splenectomy has been avoided, as both therapies expose the child to further immune compromise. Following the observation that platelet counts tend to rise after commencement of zidovudine therapy, particularly in those patients who had previously low levels (Hymes et al 1988), zidovudine has been used as a method of

treatment for thrombocytopoenia (Swiss Group for Clinical Studies 1988). Its mechanism of action is as yet unclear, but it may be related directly to anti-viral effect (Pottage et al 1988). In cases 6 and 13 resolution of thrombocytopoenia antedated commencement of zidovudine.

Cardiomyopathy

Case 2 developed a dilated cardiomyopathy at the age of 69 months, presenting acutely with symptoms and signs of left ventricular failure. Case 13, who became HIV infected as a result of surgery for congenital heart disease, has also developed a degree of cardiac failure, not felt to be related to his previous defect. Cardiac abnormalities in children with HIV infection were first reported in 1985 (Issenberg et al 1985, Sherron et al 1985), and may be found in 93% of HIV infected children; diminished contractility, suggestive of intrinsic myocardial dysfunction is found in 27% (Lipshultz et al 1989). The diagnosis of cardiac dysfunction may be made during severe systemic illness, with fever, hepatosplenomegaly, and pulmonary disease, in which case the prognosis is poor (Stewart et al 1989).

Focal myocarditis has been described, and myocardial inflammation is relatively common, but it is unusual to demonstrate a causative organism (Lipshultz et al 1989). In our case, endomyocardial biopsy was unhelpful. At present it is unclear whether HIV has a direct effect on the heart. It is also possible that zidovudine, which has been demonstrated to cause a mitochondrial myopathy (Dalakas et al 1990) has a role in the aetiology in some cases.

HIV related cardiomyopathy has been shown to respond to standard treatment for cardiac failure(Stewart et al 1989). Case 13 was controlled on diuretics, but case 2 required digoxin and captopril in addition, and despite treatment, succumbed. Death attributed to cardiac causes occurred in 4/30 children in one series (Lipschultz et al 1989).

Neoplasia

It is well recognised that tumours develop more frequently in immunosuppressed individuals. AIDS in adults is particularly associated with Kaposi's sarcoma, but although KS has been reported in children (Buck et al 1983), it is relatively uncommon. Non-Hodgkin lymphoma is 360 times more common in AIDS patients than in the general population of under 20, and primary lymphoma of the brain and Burkitt's lymphoma 1000 times more frequent (Beral et al 1991). Tumours of smooth muscle origin, and fibrosarcoma of the liver have also been reported in children with AIDS (Chadwick et al 1990, Ninane et al 1985). There have been no previous reports of Ewing's sarcoma in HIV infected children. Impaired T cell surveillance, multiple co-infecting organisms, chronic antigenic stimulation with polyclonal B cell expansion, and abnormal regulation of cytokines and growth factors may all play a role in tumour development (Levine 1989). In our case, the tumour was responsive even to a modified regimen of chemotherapy, but at the price of further immune compromise. Multicentre studies are required to determine the optimal therapeutic regimens in this difficult clinical situation. With increased survival of children with HIV infection,

it is possible that more will present with tumours in the future. A cancer registry which will include all children born to HIV seropositive women, even those who are presumed uninfected is therefore required to detect any increased susceptibility to tumour development.

Skin Disease

Cutaneous manifestations of HIV infection occurred in the majority of the children. Five had eczematous skin rashes, which have been described to occur frequently in such children (Rubinstein & Bernstein 1986) and 3 had petechiae secondary to ITP. Infectious manifestations included four with mucocutaneous candidal infection, case 2 with widespread impetigo, and case 9, with primary herpes, which in her case was uncomplicated. Two children developed widespread molluscum contagiosum, both at a time when their CD4 counts were falling. In both cases, facial papules were prominent, which is unusual in children who are not immunocompromised (Warner & Fisher 1986, Penneys & Hicks 1985). Both resolved spontaneously once T cell numbers had increased on zidovudine therapy.

Although, like the European Collaborative study as a whole, the description of early symptoms and signs of HIV infection is substantially unbiased by treatment (ECS 1991), the natural history of HIV infection in this group of children has undoubtedly been modified by therapeutic manoeuvres. This may partially account for the more prolonged survival than we had expected from early studies (Scott et al 1984, Rubinstein et al 1983). However, treatment would have to begin very early

in order to affect the prognosis of those infants developing AIDS within the first months of life. Survival of children with perinatally acquired HIV has now been described until early teenage (Gibb, personal communication), but in the absence of major advances in therapy, HIV related disease will continue to be a major cause of morbidity, and the ultimate prognosis remains bleak.

TABLE III.4.1

Clinical features of 12 HIV infected children

Case	Age * (mths)	Length of follow up (mths)	Age at 1st symptoms (mths)	Current stage	Age progressed to P2B-D(mths)	IVIgG started (mths)	AZT started (mths)
1	69	69	3	P2A	-	9	28
2	82 (D)	64	9	Dead	-	24	42
3	61	61	9	P2F	-	14	-
4	4 (D)	4	1	Dead	4	-	-
5	46	25	-	P1B	-	-	-
6	81 (D)	42	12	Dead	51	37	58
7	76	40	33	P2A	-	39-60	-
8	67 (D)	21	9	Dead	45	47	49
9	76	49	18	P2C	24	24	-
10	25 (D)	14	22	Dead	24	23	25
11	43 (D)	21	22	Dead	26	26	28
13	94	52	14	P2C	42	42	73

* as of 1.8.91 or at death (D)

TABLE III.4.2

Early symptoms and signs in children infected perinatally with HIV

Sign/symptom	No (n=11)
Recurrent URT infection	9
Recurrent diarrhoea	9
Eczematous rash	5
Petechial rash	2
Parotitis	1
Oral candidiasis	6
Lymphadenopathy	10
Hepatosplenomegaly	10
Failure to thrive	6

TABLE III.4.3

Symptoms of advanced HIV disease in perinatally infected children

Symptom	No (n=11)
PCP	2
Serious bacterial infection	2
LIP	3
Encephalopathy	3
Oesophageal candida	1
Neoplasia	1
Cardiomyopathy	1
Wasting syndrome	1

CHAPTER 5

VIRUS INFECTIONS OF THE RESPIRATORY TRACT IN HIV INFECTED CHILDREN

Aims

To investigate whether the rates of respiratory viral isolation, and severity of respiratory symptoms in HIV infected children were higher than in non-HIV infected children from the same socio-economic group.

Methods

Fifty of the children born to HIV seropositive mothers in Edinburgh were enrolled, and studied up to the age of 2 years. The five infected children (cases 1-5) formed group A. Forty five index children considered not to be infected with HIV (group B) were studied from September 1987-September 1989. Nineteen children whose fathers were HIV seropositive but whose mothers were HIV seronegative were also investigated for one year (group C).

At the three monthly visit, history and examination were performed as previously described. One set of nose and throat swabs was taken at each visit; repeat specimens taken during hospital admissions were not analysed in this study. Results were analysed using Chi-squared test for statistical significance.

Results

Three hundred and one samples were collected for investigation from sixty nine children over a 24 month period, and are summarised in detail in table III.5.1. Although a small number of opportunities for obtaining nose and throat swabs were missed, this was not significantly different between the groups. Group A had significantly more respiratory infections, and more samples positive for viral isolation than group B. Over the first year, however, groups B and C did not differ (table III.5.2).

The viruses isolated are listed in table III.5.3. There was no difference between all 3 groups in the proportion of positive virus isolations associated with symptoms. Symptoms usually accompanied infections with influenza A, parainfluenza, RS and adenovirus infection, whereas isolation of CMV or Coxsackie was more often asymptomatic, rhinovirus occurring about equally in symptomatic and asymptomatic children. CMV was isolated on more than one occasion from nose and throat swabs in HIV infected and noninfected children alike. Although upper respiratory tract symptoms accompanied this finding on some occasions, in no child was there any suggestion of CMV pneumonitis. Statistical analysis of the difference between groups was not undertaken due to the small number of viral isolations in each group. Six of the 10 proven viral infections in group A necessitated admission to hospital, compared with 9/32 in group B, a difference which was significant ($p<0.05$). All 3 children with influenza A infection were admitted. Others were RSV (3 group B), parainfluenza (2 group A, 1 B), adenovirus (2 A, 1 B) and measles (1 B). No child required assisted

ventilation, and specific antiviral therapy was not required.

The three children from group A who suffered from recurrent infections were commenced on IVIgG infusions, as prophylaxis against recurrent bacterial infections as described in chapter 9. In these 3 children, the proportion of virus isolation positive specimens before treatment was initiated was 7/11, compared with 3/13 in the subsequent period while they were receiving regular IVIgG infusions, a decrease which also was significant ($p<0.05$). No child was commenced on zidovudine during this time.

In one child in group B, influenza A infection was associated with a profound drop in CD4 count, from 2.04 x 10^9/l to 0.27 x 10^9/l, and in CD4/CD8 ratio form 1.9 to 0.5, returning to previous levels on recovery from the illness. We therefore analysed the effects of respiratory symptoms and viral isolation on mean CD4 counts for groups A and B. No significant difference in mean values were observed between samples taken when asymptomatic and at the time of symptoms or viral positivity for either group, although the mean was lower in all cases in infected children (0.99 x 10^9/l in group A and 2.69 x 10^9/l in group B). Similarly, no change was seen in CD8+ lymphocyte levels.

Discussion

The susceptibility of HIV infected children to recurrent and chronic bacterial respiratory infection is coincident with a functional antibody deficiency (Pahwa et al 1986). As HIV infected children also have a

cellular immunodeficiency, it might therefore be anticipated that these children may suffer more acute viral infections (as in Severe Combined Immunodeficiency) than HIV uninfected children.

Despite the small number of children proving HIV infected available for study, we have demonstrated an increased rate of respiratory viral isolation in HIV infected children in the first two years of life compared with appropriate controls which reaches statistical significance. Although it may at first appear that the infected group were sampled more often (6.0 times per child in the infected children compared with 4.9 times per child in the uninfected children), this is due to the differing ages of the children at the start of the study, and some children not having reached 2 years at the close of the study. Neither the number of missed sampling opportunities nor the frequency of sampling differed between the 2 groups (table III.5.1). The increased rate of viral isolation observed may be due to HIV infection causing greater susceptibility after exposure to viruses other than HIV, or to a greater virus load and more prolonged excretion during any given episode, making viral isolation on sampling at intervals more likely. We have not, however seen the chronic excretion of agents such as parainfluenza virus, which has been reported by others (Josephs et al 1988).

A recent study has suggested that HIV infected children who develop RSV bronchiolitis have prolonged viral carriage, and a high mortality rate of 20% (Chadwani et al 1990). However, both the deaths in the study by Chadwani et al occurred in children who were severely immunocompromised (CD4 counts less than $0.2 \times 10^9/l$) and had

superimposed opportunistic infection. It is perhaps fortuitous that our HIV infected children escaped RSV infection when RS infections were widely occurring in the community, but the uncomplicated course of other viral infections, such as parainfluenza suggests that their immune function was relatively well preserved at the time, as these children had a mean CD4 count of 0.99 x 10^9/l.

In our study we used two different control groups of children who had the same social background as the HIV infected children, and have demonstrated that children born to HIV seropositive mothers who are not HIV infected do not differ in rates of respiratory infection from those not exposed to the risk of HIV infection. In all three groups poor housing, overcrowding, and parental smoking habits may contribute to a higher than average morbidity in this respect.

The finding of fewer positive viral isolates in HIV infected children following IVIgG therapy is interesting, although given the small number of children involved, these results should be interpreted with caution. One possible explanation could be that children outgrow susceptibility to viral infection after the first year of life, but this is not borne out by the rates in group B children, which were constant between the two years. Nonetheless, immunoglobulin therapy has been shown to prevent Echovirus infection (Nagington et al 1983), reduce the shedding of RSV (Hemming et al 1987), and may have beneficial effects in CMV infection (Morris 1988 [b]), suggesting that the reduction of viral isolates may be a beneficial side effect of IVIgG therapy.

We conclude that respiratory viral infections occur more

frequently in HIV infected children. Given the ubiquitous nature of these agents and the lack of available specific therapy for treatment or prophylaxis, further studies are required in larger groups of HIV infected children to assess the incidence of recurrent viral infections and their influence on the progression of HIV disease together with the role of immunotherapy, such as the early use of IVIgG.

TABLE III.5.1

Virus isolation in HIV infected (group A), and HIV uninfected (group B) children born to HIV seropositive women

	Group A	Group B	p*
no. of patients	5	45	
no. of samples	30	220	
age range (mean, median)	3-24(14.5, 15)	3-24(14.0, 15)	NS
no. of missed sampling opportunities (%)	4/34 (12%)	22/242 (9%)	NS
no. of symptomatic resp. infections	14	41	<0.01
no. of positive virus isolations	10	32	<0.05

* using the Chi-squared test

TABLE III.5.2

Virus isolation in uninfected children born to HIV seropositive (group B) and HIV seronegative (group C) women

	Group B	Group C	p
no of patients	30	19	
no. of samples	104	51	
age range (mean, median)	3-12(7.5, 9)	3-12(7.9, 9)	NS
no. of missed sampling opportunities	11/115 (9%)	6/57 (11%)	NS
no. of symptomatic episodes	13	5	NS
no, positive virus	15	8	NS

TABLE III.5.3

Viruses isolated from symptomatic and asymptomatic children born to HIV seropositive women

Virus	Symptomatic		Asymptomatic		Total
	A	B	A	B	
Adenovirus	2	2	0	1	5
Influenza	0	3	0	0	3
Parainfluenza	2	2	0	1	5
RSV	0	3	0	1	4
Rhinovirus	1	4	0	3	8
CMV	3	1	2	5	11
HSV I	0	1	0	1	2
Coxsackie B	0	0	0	2	2
Measles	0	1	0	0	1
Echo	0	0	0	1	1
Total	**8**	**17**	**2**	**15**	**42**

CHAPTER 6

DIAGNOSIS, TREATMENT AND PROPHYLAXIS OF PNEUMOCYSTIS CARINII PNEUMONIA

Aims

To describe the diagnosis of PCP from non-invasive sampling of respiratory secretion, and to assess the impact of the new CDC guidelines for PCP prophylaxis on our cohort.

Methods

Laboratory diagnosis of PCP

Following addition of an equal volume of sputolysin (Behring Diagnostics) and glass beads the sample obtained from nasopharyngeal aspiration was vortexed for one minute and then incubated at 37^{O}C for three minutes. Ten ml of phosphate buffered saline pH 7.2 was added and the mixture centrifuged at 2500g for 20 minutes. The pellet was resuspended in 1ml PBS and cytospin preparations made on two poly-lysine coated slides. A cytospin preparation of positive rat lavage material was used as a positive control. After fixation for 30 minutes in equal volumes (50%) methanol and acetone, trypsin (0.25%) digestion for ten minutes at 37^{O}C followed. A standard wash procedure of three five minutes with PBS was carried out.

Mouse monoclonal antibody to P. Carinii (Northumbria Biologicals Limited) was added to the test and positive control slides and PBS only, and incubated for 40 minutes. After repeat washing antimouse IgG (F`ab) conjugated to fluorescein isothiocyanate was added and a further incubation of 40 minutes followed. After a last wash procedure substituting PBS pH 8.4 for the final change, the slides were mounted and viewed under UV light.

The sample was considered positive when three typical cysts showing all-over fluorescence were seen, further confirmed by methenane silver treatment of the control slide.

2) Analysis of CD4 counts

Routine measurement of CD4 counts became available in 1987. All samples obtained from the study population after this time were used.

Results

Two children in our series had proven PCP. In case 11 PCP was the terminal illness, and diagnosis was made at autopsy by silver staining. In case 4, described in appendix II, the diagnosis was successfully made from nasopharyngeal secretions using the fluorescent antibody technique described above.

The ranges for CD4 counts within the uninfected children are

shown in table III.6.1. CD4 counts in cord blood showed a wide variation, and 9/42 samples from non-infected and control children were CD4 lymphopoenic ($<1{,}000 \times 10^6$/l). Case 4 is the only infected child on whom cord blood data are available, but she had a normal count of $2{,}340 \times 10^6$/l at birth. At 3 months, when she already had candidiasis, her CD4 count, at $1{,}940 \times 10^6$/l, was still outwith the recommended level for prophylaxis (CDC 1991), and was only found to have dropped further to $1{,}080 \times 10^6$/l when she presented with PCP. Two index children who later proved uninfected had CD4 counts less than $1{,}500 \times 10^6$/l in the first year of life, and so would have been treated had these guidelines been available, and one control child had a similarly low CD4 count.

Of the other HIV infected children, 5 had CD4 counts sufficiently low to merit prophylaxis (CDC 1991). Of the three who received none, case 11 developed PCP, but at a stage when he was otherwise grossly debilitated, with an extremely poor quality of life. Two children, cases 6 and 13 received prophylaxis, and neither developed PCP. Case 6 failed to tolerate cotrimoxazole and was commenced on monthly aerosolised pentamidine when his CD4 count dropped below 200×10^6/l, in accordance with adult guidelines. Case 13 was commenced on oral cotrimoxazole 3 times weekly at the time of diagnosis of HIV infection. No side effects were experienced by either child.

Discussion

In HIV infection, the diagnosis of PCP has important implications, not only for the treatment of the acute illness, but also for

longer term prognosis. In general, children present with moderate to severe disease and significant hypoxia, as did case 4 (Bernstein et al 1989). Overall mortality due to PCP is quoted at 31%-70% in different series (Kovacs et al 1991, Bernstein et al 1989, Connor et al 1991), but in infants less than 6 months old, mortality has been reported as high as 87% during the acute episode, and 100% by 1 year (Chow et al 1989). Because of the risks involved in, and the technical expertise required for paediatric bronchoscopy, treatment is usually empirical. If the child succumbs, autopsy may give the answers, as in case 11, but if the child responds to high dose cotrimoxazole, the differential diagnosis between a bacterial pneumonia and PCP remains unresolved. This then creates difficulties in classification and staging of the child's disease, and in making therapeutic decisions, such as whether to commence zidovudine or PCP prophylaxis.

Identification of PCP from upper respiratory tract secretions may only be possible in cases of overwhelming infection, and failure to detect PCP by this method does not exclude the diagnosis. However, it is in the most severe cases that the patient is least able to tolerate more invasive procedures. Because our technique is rapid, other methods can still be used thereafter without undue delay. We believe therefore that it should be used as the first line investigation of suspected PCP.

Case 4 was treated promptly with high dose cotrimoxazole. In adults, this has been reported to be at least 80% effective in treatment of first time episodes, being equally effective in this respect as intravenous pentamidine (Corkey et al 1988), and it remains first-line

treatment. There is a high incidence of hypersensitivity reactions to the sulphonamide, and myelosuppression may occur(Wofsy 1987), but case 4 escaped these sequelae. Intravenous pentamidine may also cause neutropoenia, and tissue accumulation in the liver, kidney, adrenal and spleen than in the lung, producing toxic effects in these organs(Donnelly et al 1988). Aerosolised pentamidine has been shown to produce higher levels in the lungs with little systemic absorption, and may become the treatment of choice in the future(Montgomery et al 1988). Case 4 died before alternative treatment was considered. It has since been suggested that early use of corticosteroids may prevent deterioration (Montaner et al 1990), but there was no experience of their use in infants at this time. Survival following ventilation for PCP in children has been described (Connor et al 1991), but the prognosis for children requiring mechanical ventilation is extremely gloomy, such that the justification for this course of action must be severely questioned(Vernon et al 1988).

Our ranges for CD4 counts by age agree with other series (Denny et al 1990). If the CDC guidelines for PCP prophylaxis had been followed case 4 would not have received prophylaxis before presenting with PCP, but 2 children who subsequently proved HIV uninfected would have been treated unnecessarily. More studies are therefore required to identify those children at particular risk of developing this severe early consequence of HIV infection. In infants under a year in whom a definitive diagnosis of HIV has been made, particularly in the presence of candidiasis (ECS 1991) there is a case for universal prophylaxis irrespective of CD4 count.

Current CDC recommendations for the choice of prophylactic

agents are listed in Appendix I, table 7. It remains to be seen whether these measures will have an impact on the mortality from HIV infection, or whether other manifestations of immune compromise will merely assume a more prominent role in causing morbidity and mortality.

TABLE III.6.1

CD4 counts from HIV uninfected children.

	n	mean CD4	SD	SE	*
			$x10^9/l$		
Cord	42	1.8	1.11	0.17	1.5
3 months	35	3.32	0.86	0.15	1.5
6 months	31	3.37	1.12	0.2	1.5
1 year	46	2.65	0.93	0.14	0.75
18 months	52	2.32	0.73	0.09	0.75
2 years	53	2.02	0.77	0.1	0.5
3 years	43	1.48	0.6	0.09	0.5
4 years	34	1.3	0.55	0.09	0.5
5 years	23	1.09	0.49	0.1	0.2

* CDC recommended level for starting PCP prophylaxis

CHAPTER 7

THE PROGNOSTIC VALUE OF HIV ANTIGEN, CORE ANTIBODY, CD4 LYMPHOCYTE AND IMMUNOGLOBULIN MEASUREMENT - A LONGITUDINAL STUDY

Aims

This study set out to quantify HIV Ag, p24 Ab levels, and measure CD4 lymphocyte counts and immunoglobulin levels, to relate these to the onset of symptoms, clinical progression and therapeutic intervention.

Patients and Methods

Cases 1-11 were identified at age 0-47 months (median 21 months). Three had been followed since birth, two were identified while still asymptomatic, and 6 presented with symptomatic disease. The median duration of follow-up was 36 months (range 4 - 69). In those surviving, the median follow-up was 51 months (range 24-69).

Results

Of the 11 children, 10 had symptoms and signs of HIV infection, one child (case 5) had lymphadenopathy, hypergammaglobulinaemia (IgG of 20g/l) and reversed CD4/CD8 ratio (0.8), but remained clinically well. Nine children had detectable HIV Ag during follow-up, and in 5, HIV Ag was detected before the onset of symptoms. Clinical

details can be found in appendix II and are summarised in table III.4.1, p111). Laboratory data is contained in table III.7.1. In order to illustrate the different patterns in laboratory indices with time, sequential HIV Ag, P24 Ab, CD4 counts and weights on all 11 children are illustrated in figs III.7.1-11. The 50th centile for weight is that defined on standard Tanner charts, and that for CD4 count is derived from table III.6.1.

Six children (cases 2,4,6,8,10,11) had HIV Ag levels above 50pg/ml and were negative for p24Ab (group 1). All died at ages 4-82 months, median 45 months. Four (cases 1,3,7,10) had low or negative HIV Ag values, and none has died nor progressed to AIDS during the period of follow up of 40-69 months (group 2). Of these, 2 have been negative for p24 Ab, but all now have detectable p24Ab. Two children (cases 5 and 6) have been positive for p24 Ab, but also HIV antigenaemic (group 3). Both remained clinically stable while positive for both HIV Ag and p24 Ab. Case 6 subsequently became negative for p24 Ab, meeting the criteria for inclusion in group 1 and so he changed group at this point. Clinical deterioration ensued. Case 2 was HIV Ag negative for 30 months, during which time her clinical condition was stable, and only developed HIV Ag >50pg/ml at 72 months of age, after which she is included in group 1. Group 1 children also showed a significantly greater rate of fall in CD4 count than group 2 ($p<0.01$, Wilcoxon rank sum test). CD4 count remained stable in cases 5 and 6 while they met group 3 criteria.

Data are available on 6 children in the first 2 years of life, and 3 were followed from birth. Case 4 was negative for p24 Ab from birth, with rapidly increasing HIV Ag to 145pg/ml at her death at the age of 4

months. Cases 1,2, and 3 had positive values for p24 Ab initially, but levels declined, coincident with emergence of low levels of HIV Ag. In these children, who were clinically stable, HIV Ag became negative and p24 Ab was subsequently detected.

CD4 counts are available for 6 children at the onset of symptoms. The mean count was 3.02×10^9/l, which is well within the normal range (see table III.6.1, page 125). Although in most cases CD4 counts only began to fall once clinical symptoms and signs were apparent, case 10 remained clinically well for 6 months after her CD4 counts had dropped precipitously.

Table III.7.1 also shows the rate of decline of CD4 counts for each child. Those who died had a greater rate of decline per year (median 90%, range 25%-99%) than those who remained stable (median 15%, range 0%- 25%). This is true even for case 4 who died with a CD4 count of 1.08×10^9/l, as discussed previously. Four of the other children who died had unrecordable counts in the terminal phase. A CD4 count persistently less than 0.1×10^9/l was associated with death after a median of 6 months (range 3-8 months).

Raised IgG levels above the normal range for age were seen in all children reaching 6 months of age; by 6 months in cases 1 and 3 followed from birth, and at presentation in all others. Levels rose to a median peak of 28g/l (range 4-72g/l), at the age of 47 months (range 4-63). Nine children also had raised IgM, reaching a median peak of 3.4g/l (range 2.25-5g/l) at 51 months (range 15-82). There was no relationship between either IgG or IgM levels and progression of

disease. Four children (cases 6, 8, 10, 11) had raised IgA levels. In all cases levels rose before progression of clinical disease, and was associated with a poor prognosis. All children had died within 2 years of IgA being above the normal range.

The effect of IVIgG and AZT on laboratory parameters is discussed in subsequent chapters. Cases 6 and 8 received plasma rich in anti p24 antibody on 2 and 6 occasions respectively. Both had at this time received IVIgG infusions for 18 months. One child (case 8) had received zidovudine in the past, but this had been discontinued because of toxicity and poor compliance. She was extremely ill with unresolving pneumonia, and died 8 weeks later. Case 6 remained clinically stable during treatment. Figure III.7.12 shows the serological response in case 6. The plasma infusions were effective in clearing HIV Ag completely, and raising p24 Ab levels 2 hours after completion of the infusion. However, as p24 Ab levels declined, HIV Ag reappeared between 7 and 14 days later, returning to the previous level by 6 weeks. On the 3 weekly infusion regimen HIV Ag reemerged despite positive p24 Ab. Children in group 1 treated with IVIgG and AZT, they still had a poorer prognosis than children in group 2.

Discussion

Our results indicate that HIV Ag assay is valuable as an early diagnostic test in children born to HIV seropositive mothers. Nine of eleven infected children were positive for HIV Ag, 6 of whom were at the time either asymptomatic or had symptoms not specific for HIV infection. Our study also underlines the importance of regular

prospective follow up of all children born to HIV infected mothers, as peak HIV Ag levels may occur in the asymptomatic phase, and would be missed if the child were only identified on becoming ill.

Other studies have correlated persistent HIV antigenaemia and loss of p24 Ab with a poor prognosis(Epstein et al 1988 [b], Ellaurie & Rubinstein 1991). Our results also suggest that high levels of HIV Ag in the absence of p24 Ab rather than the duration of antigenaemia is significant in HIV infected children whose disease progressed. Our observation of the difference in the rate of decline of CD4+ lymphocytes between groups 1 and 2 has been described in adults (Andrieu et al 1988), but there has been no previous report in children.

A decline in p24 Ab has been reported to precede HIV antigenaemia, and to correlate with a poor prognosis in adults (Forster et al 1987). In children born to HIV seropositive women, such a finding is difficult to interpret because of the inevitable decline of passive maternal antibody in the first year of life. There have been no previous reports relating p24 Ab levels at birth with progression of disease in children, although our data, and others' suggest that maternal p24 Ab during pregnancy does not correlate strongly with the risks of transmission (Goedert et al 1989). Our preliminary longitudinal study of three children suggests that absence of maternal p24 Ab at birth may be associated with rapid progression in children who are infected, but further studies on larger groups of children are required.

Two of the children studied (group 3) were simultaneously p24 Ab positive and HIV antigenaemic. In 2 previous reports (Borkowsky et al

1989, Ellaurie & Rubinstein 1991) this feature was found in half of the children studied, but no attempt was made to correlate this finding with prognosis. In adults it occurs infrequently (Andrieu et al 1988). This may be because high levels of antibody are produced as a response to HIV Ag by the immune system of the child. Children may also be more likely to have active viral replication, and the rate of spontaneous mutation of HIV is such that the p24 Ab produced by the host lacks specificity, and so avidity. It is interesting, however, that in the children we studied this state was not associated with rapid clinical deterioration. In case 6, clinical status and CD4 counts remained stable until p24 Ab became undetectable.

The association between declining CD4 count and progression to opportunistic infections and AIDS is well documented in adults (Masur et al 1989, Philips et al 1989, Schecter et al 1987). In children, interpretation of falling CD4 counts is complicated by the fall that normally occurs within the first years of life (Falcao et al 1987), but our findings that low CD4 counts of $<0.1x10^9/l$ and a rate of decline of >60%/year are associated with a poor prognosis supports the findings of other groups (Blanche et al 1990, Monforte et al 1990). The rate of decline of CD4 numbers, rather than the absolute number, may provide a useful guide as to when to commence therapy, rather than waiting for symptoms to develop.

The usefulness of immunoglobulin measurement in the first months of life has been discussed in chapter 2. We have found no correlation between the levels of IgG and progression of HIV disease, although very high levels may lead to symptoms of hyperviscosity (see chapter 9). The association between IgA levels >4g/l and shortened

survival is well documented in adults (Pederson et al 1990), but is not thought to be as good a predictor of development of AIDS as CD4 counts or B2 microglobulin and neopterin (Fahey et al 1990). Its predictive value in children has yet to be explored.

The use of plasma rich in p24 Ab has not been reported in children, although clinical benefit has been described in adults (Jackson et al 1988). We therefore chose a dose of plasma equivalent to that which had already been used in Jackson's study, and continued as long as case 8 survived, and as long as supplies lasted in case 6. Little is known about the role of host immunity in HIV infection, but if HIV antibody has a protective role then passive HIV specific immunisation may have a place once humoral immunity becomes defective. If HIV Ag itself is a direct cause of symptoms or progression in HIV infection, a reduction of HIV Ag levels may be beneficial (Jackson et al 1988). We demonstrated that infusion of this plasma rich in p24 Ab was effective temporarily in removing circulating HIV Ag. However, the effect was short-lived, HIV Ag reappearing in 2 weeks. Further studies are required to determine the dosage, volume and frequency of administration necessary for sustained antigen suppression before clinical effects can be evaluated.

We conclude that HIV antigenaemia may precede symptoms in HIV infected children, when CD4 counts are well maintained, and thus HIV Ag may be a more useful aid to early diagnosis. High levels of HIV Ag, loss of p24 Ab, and low or rapidly declining CD4 counts are poor prognostic signs irrespective of current clinical status, or therapeutic intervention.

TABLE III.7.1

Laboratory data on the 11 HIV infected children

Case	1st sample available(mths)	1st HIV Ag+ (mths)	Peak Ag (pg/ml)	1st p24Ab -ve (mths)	1st CD4 count ($x10^9$/l	Last CD4 count ($x10^9$/l)	% decline CD4/yr	Age IgA first raised (mths)	Peak IgA (g/l)
1	3	6	11	6	2.67	0.66	15%	-	-
2	18	23	79	24	2.72	0.2	25%	-	-
3	3	3	21	12	1.61	0.68	18%	-	-
4	0	2	145	0	2.34	1.08	90%	-	-
5	21	21	>150	-	0.83	1.46	0%	-	-
6	42	42	>1000	49	3.45	0.01	60%	62	9.15
7	36	-	0	-	1.11	0.69	25%	-	-
8	47	47	>150	47	0.09	0.01	95%	44	6.5
9	24	-	0	-	0.88	0.56	10%	-	-
10	12	15	>150	12	2.14	0.01	99%	21	3.6
11	21	24	97	24	5.5	0.01	88%	24	4.5

FIGURE III.7.1

Laboratory parameters for case 1

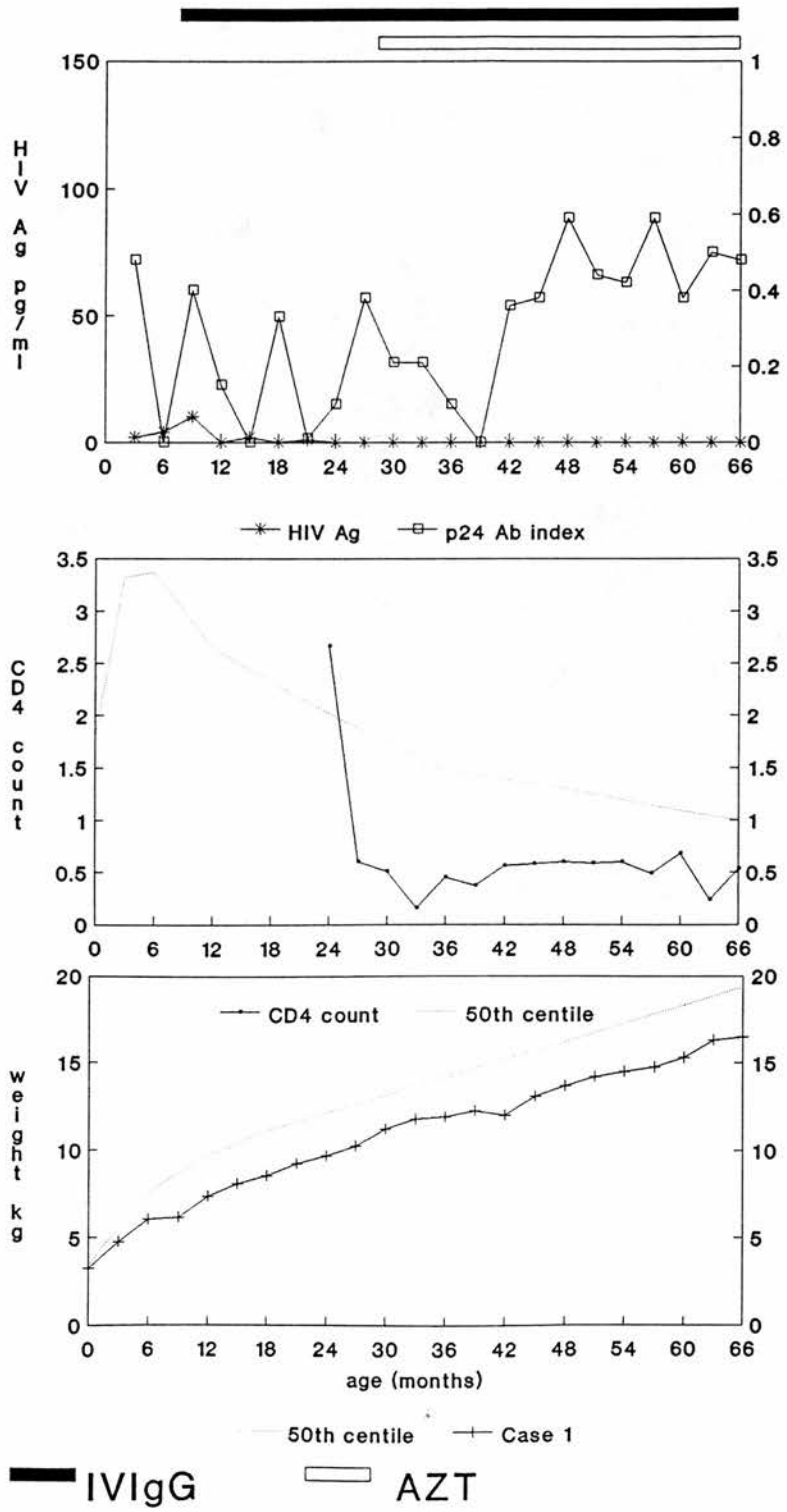


FIGURE III.7.2

Laboratory parameters for case 2

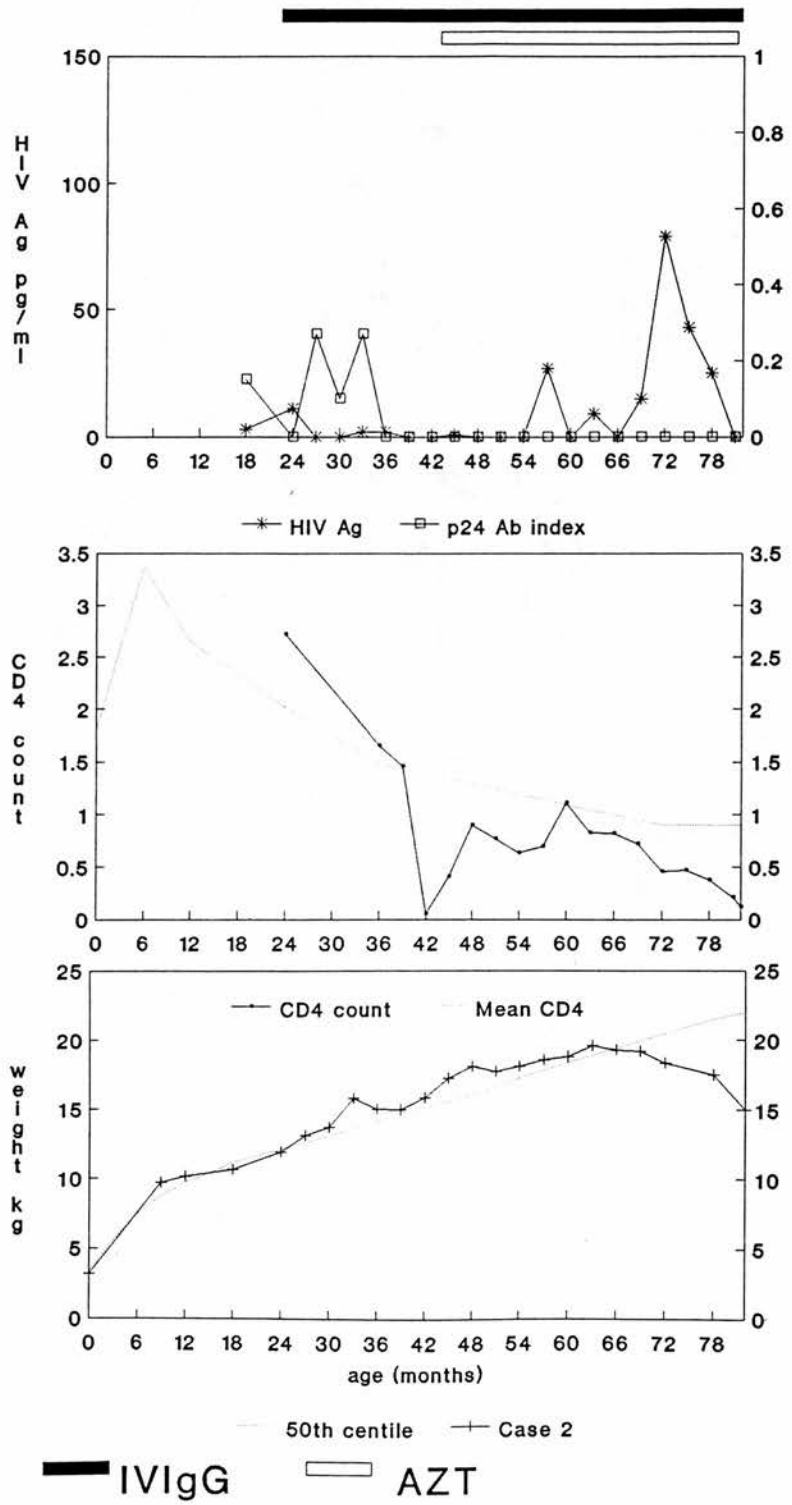
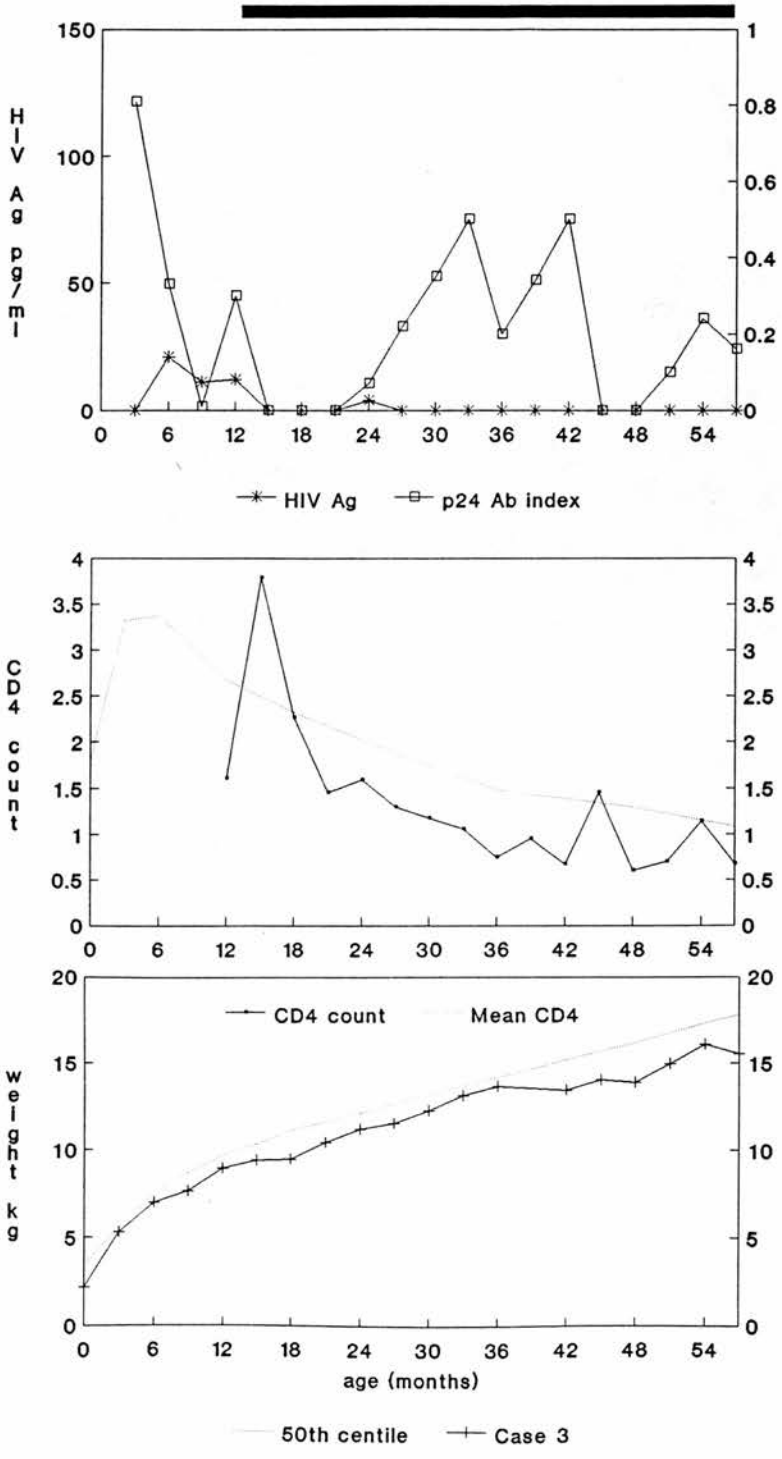


FIGURE III.7.3

Laboratory parameters for case 3



IVIgG

FIGURE III.7.4

Laboratory paprameters for case 4

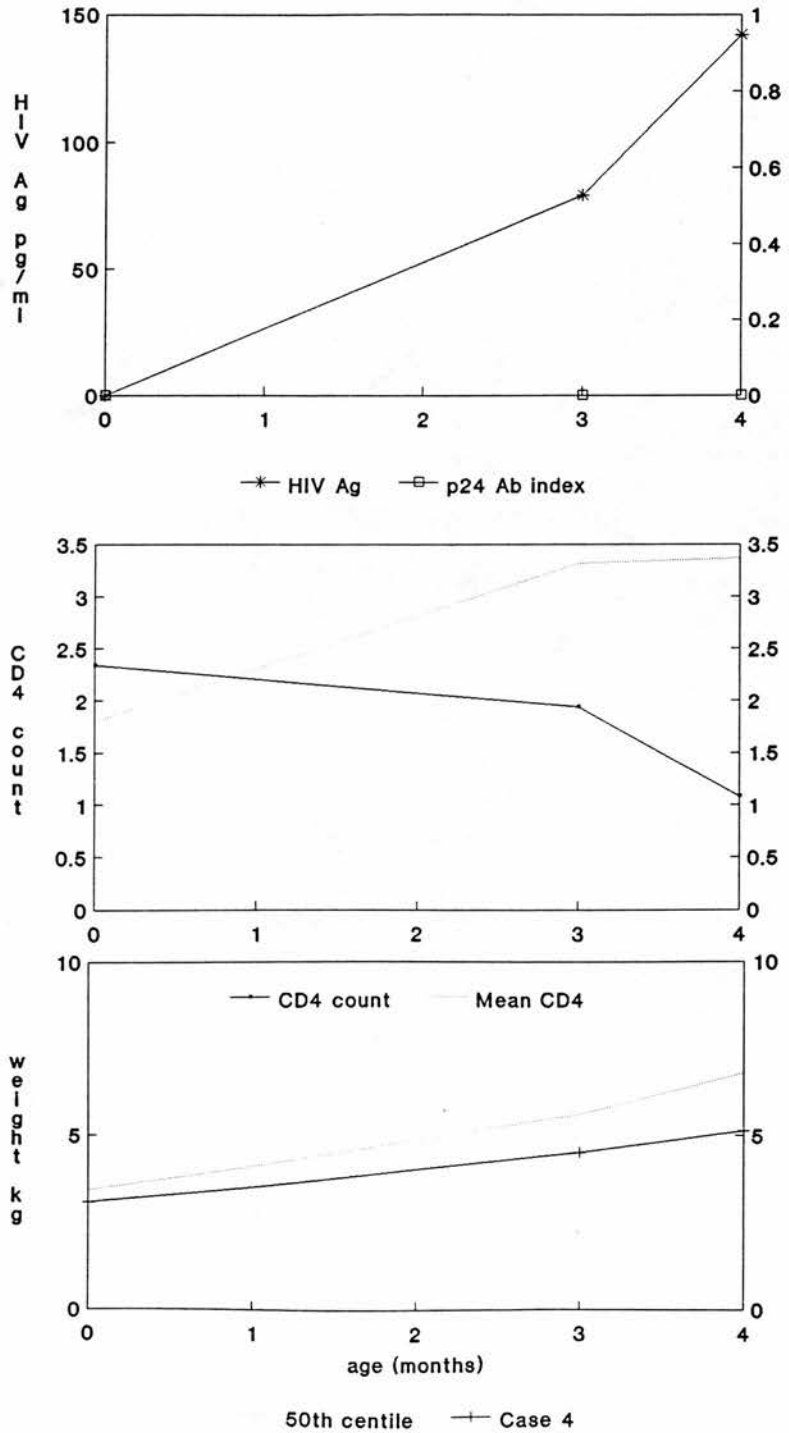


FIGURE III.7.5

Laboratory parameters for case 5

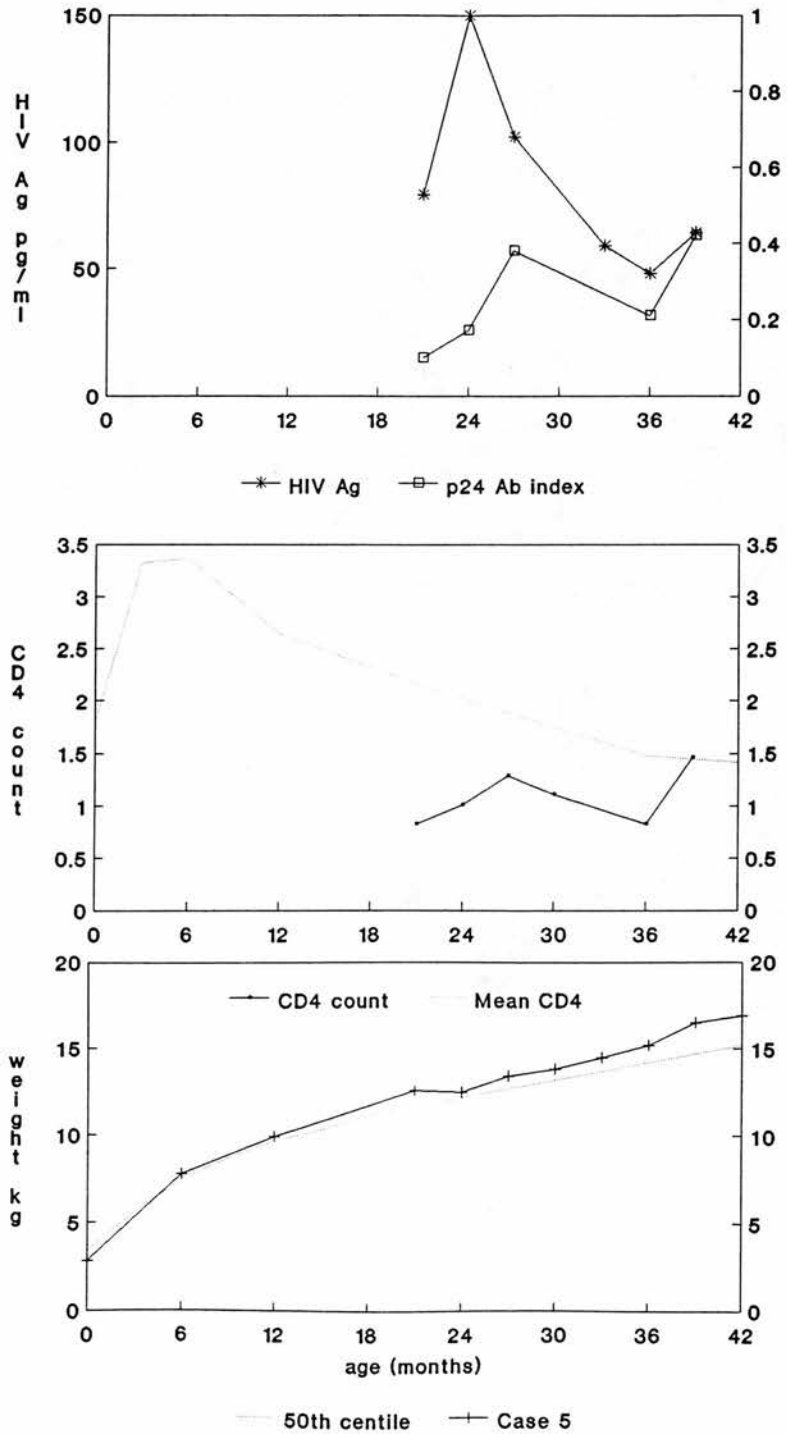


FIGURE III.7.6

Laboratory parameters for case 6

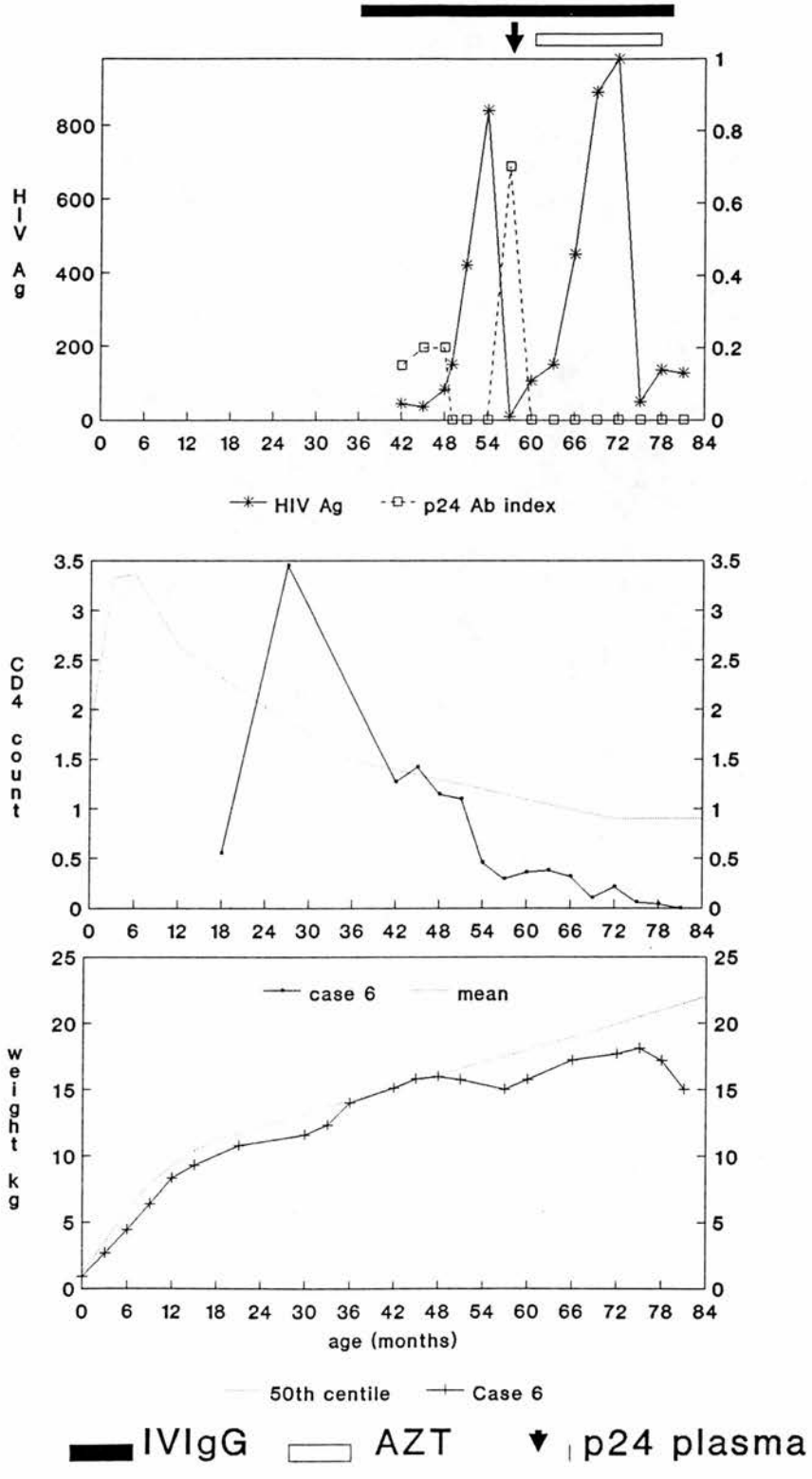
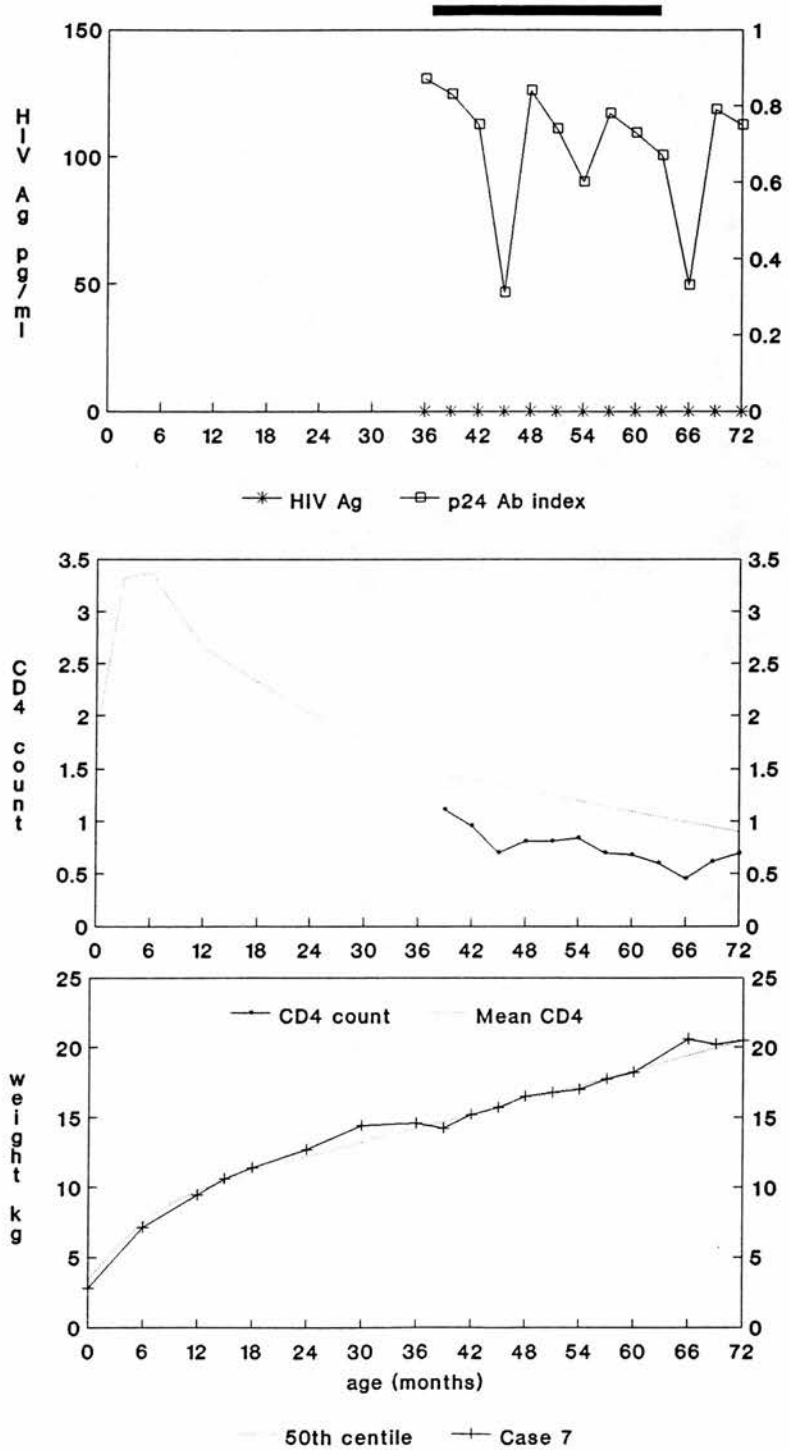


FIGURE III.7.7

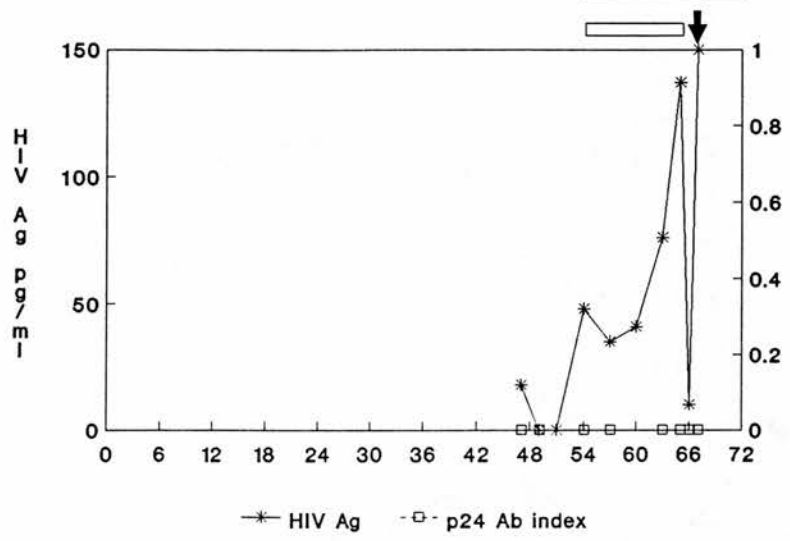
Laboratory parameters on case 7

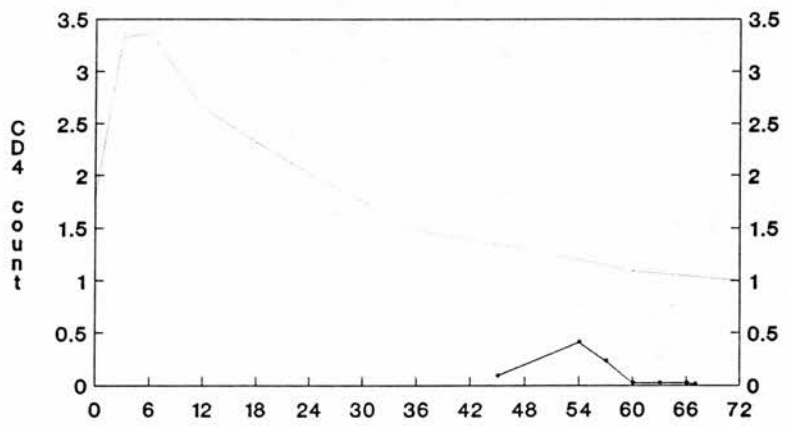


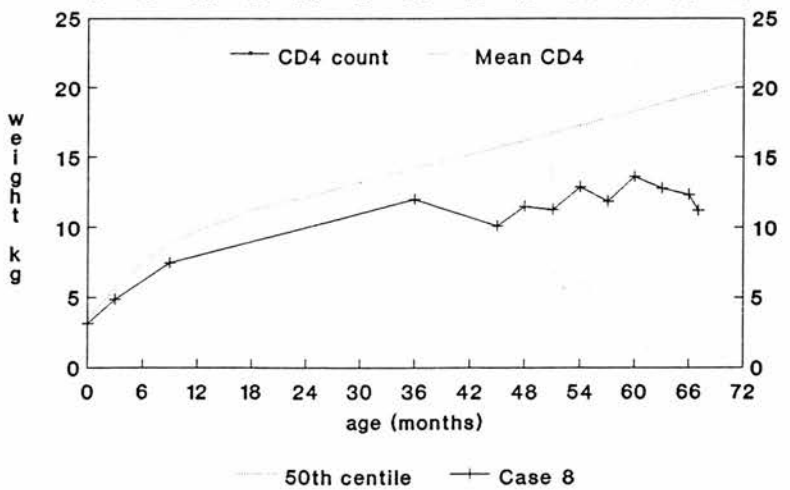
IVIgG

FIGURE III.7.8

Laboratory parameters for case 8



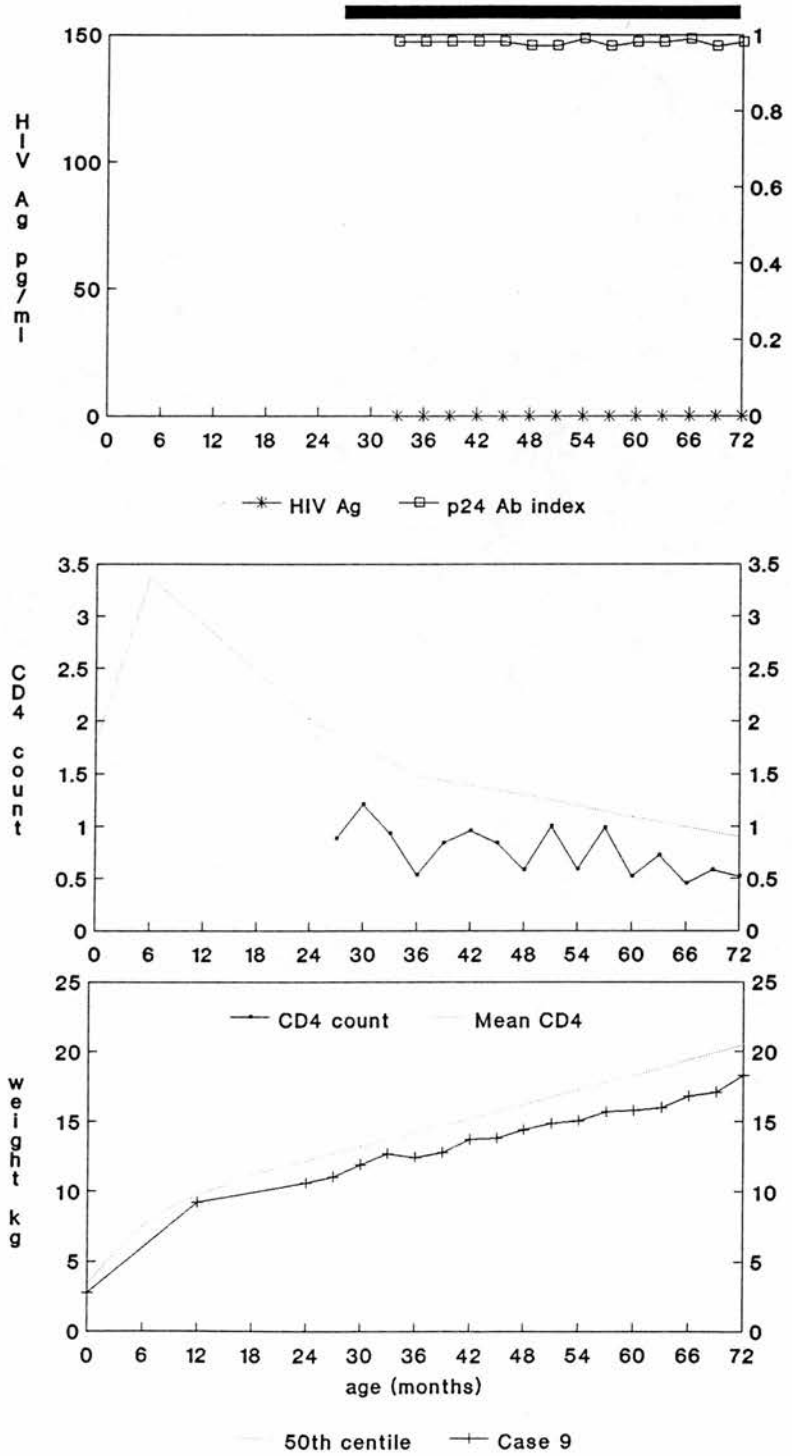




IVIgG AZT p24 plasma

FIGURE III.7.9

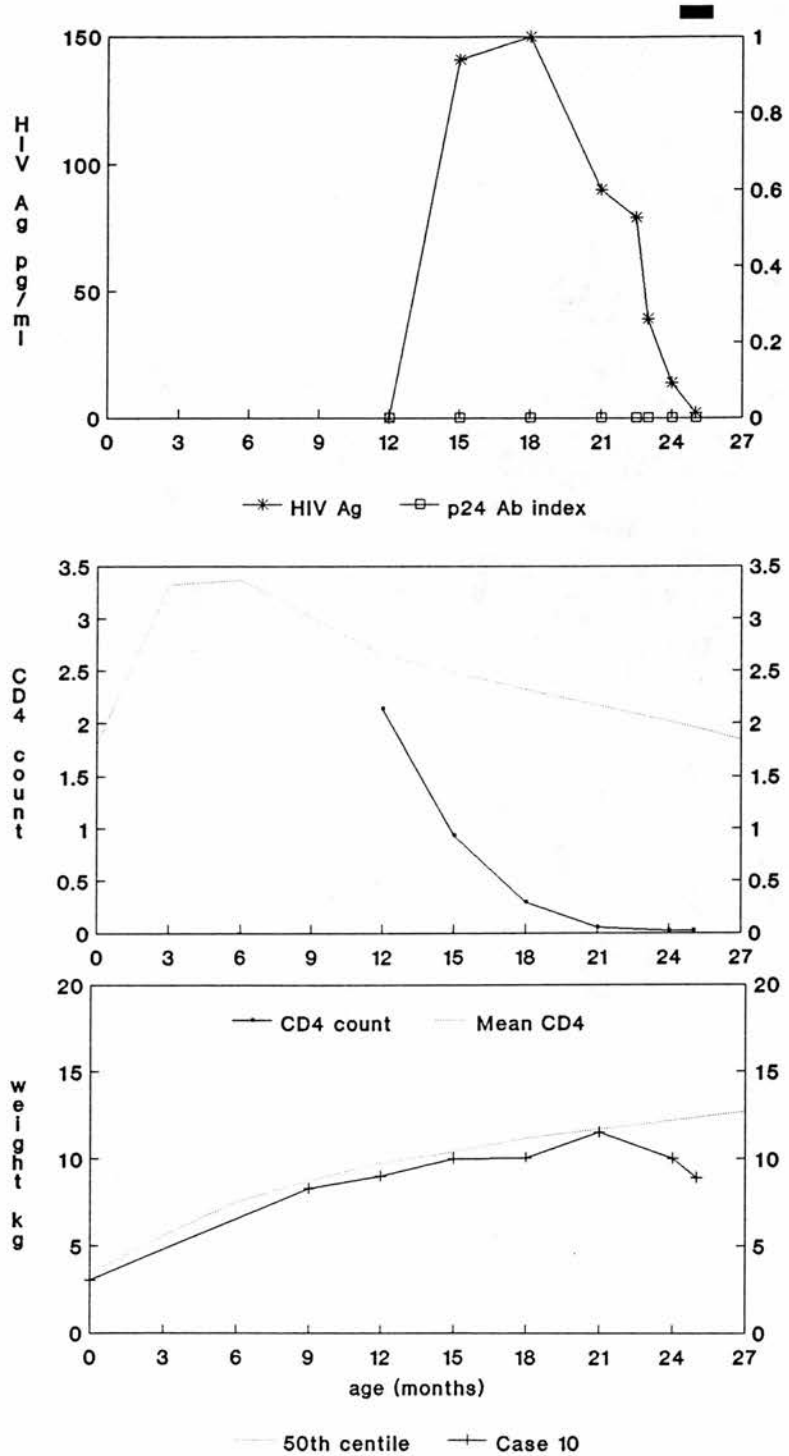
Laboratory parameters for case 9



IVIgG

FIGURE III.7.10

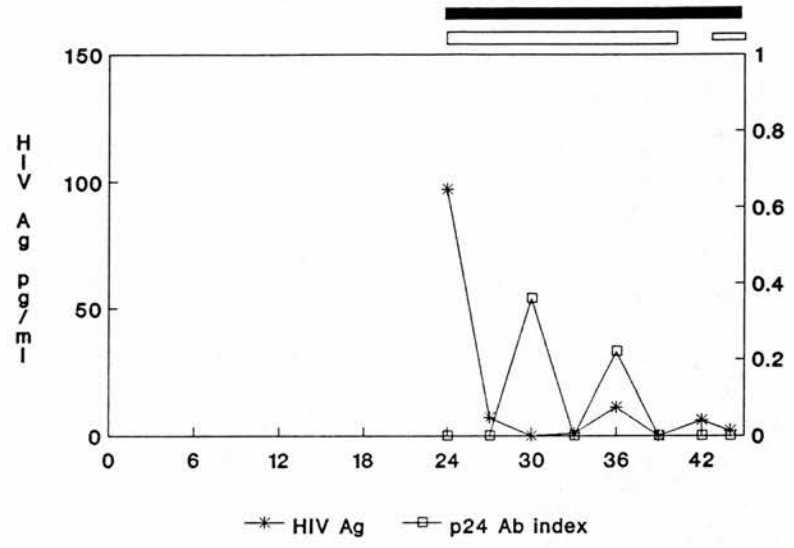
Laboratory parameters for case 10

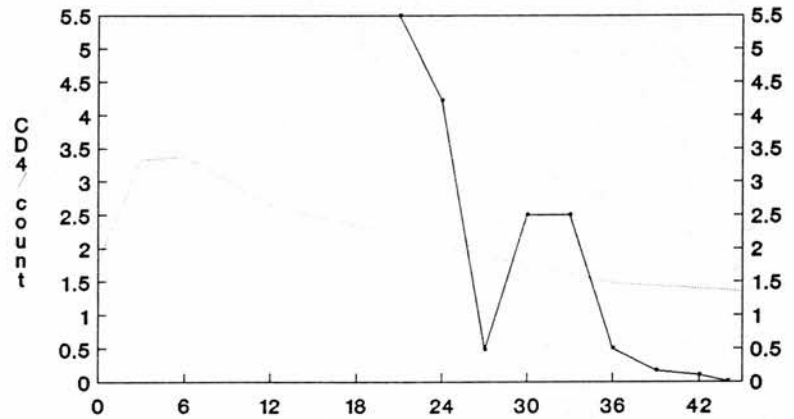


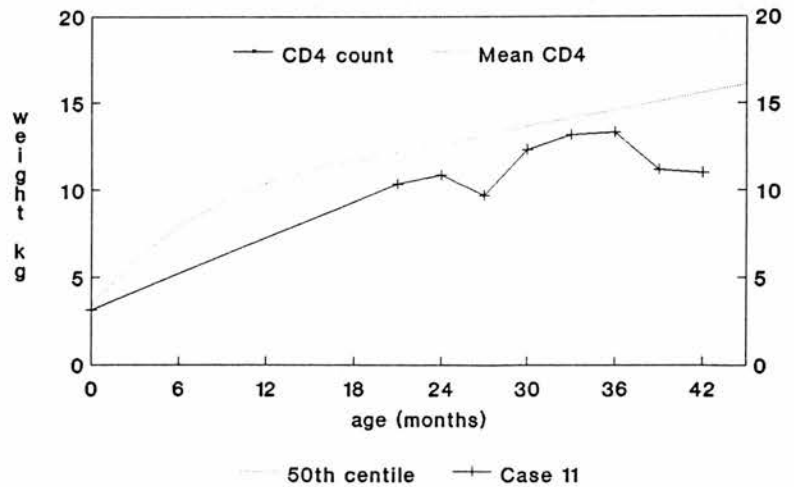
IVIgG

FIGURE III.7.11

Laboratory parameters for case 11

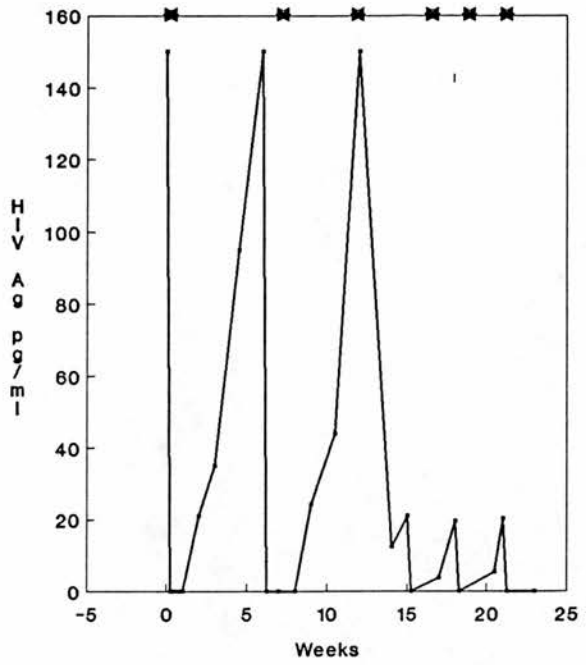




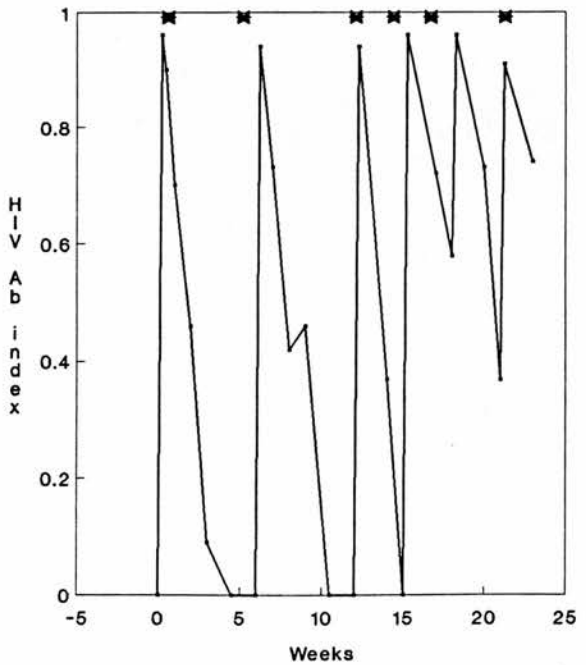


IVIgG AZT

FIGURE III.7.12



anti p24 plasma infusion



HIV Ag, p24 Ab levels during anti p24 plasma infusions

CHAPTER 8

HLA ANTIGEN FREQUENCIES IN CHILDREN BORN TO HIV INFECTED MOTHERS

Aims

This work represents a preliminary study to look for HLA associations in the study children.

Patients and methods

The subjects were 48 infants from the Edinburgh cohort and also cases 6-10. Whenever possible, tissue typing was performed immediately after birth from cord blood; otherwise samples were obtained during follow-up visits. Tissue typing for HLA-A, B, and DR antigens was carried out as previously described (section II.3) and control data from unselected babies born to healthy mothers were taken from a previous study (Jazwinska & Kilpatrick 1987).

Results

The HLA antigen frequencies obtained from 47 unrelated babies are listed on tables III.8.1 and III.8.2. The frequencies for the total group under study differed little from those of the control group born to healthy mothers (unselected for HIV status). However, there were marked increases in frequencies of HLA-B18 ($RR=7.9$; $x^2=7.9$; $p=0.005$), B7 ($RR=1.9$; $p>0.05$) and DR2 ($RR=2.0$; $x^2=3.74$;

p=0.05). The associations with HLA-B18 and DR2 were not prior hypotheses, however, and so are not statistically significant after correction for the number of antigens tested.

Comparing HLA antigen frequencies between HIV infected and uninfected babies, the most striking difference was in HLA-DR3, which was 3-fold higher in the infected infants. Frequencies of HLA A1 and A2 were also elevated in the infected group, while HLA-A3 was proportionately more abundant in the HIV uninfected group.

The rather high frequencies of both B7 and DR2 prompted us to examine the second commonest HLA antigen combination in the general population, A3, B7, DR2, as well as the much commoner A1, B8, DR3 combination which has formerly been implicated in disease susceptibility by others (table III.8.3). The combination A3, B7, DR2 (which often occurs as a haplotype) was four times commoner in our study population relative to the controls (RR=3.9; x^2=9.1; p<0.003), but was found only in babies who were not HIV infected. The combination A1, B8, DR3, in contrast, was found less often than expected in our study group (RR=0.39; p=0.06) and was disproportionately represented amongst the infected babies.

Five families were also studied (one member of each was included in the unrelated series). There were three sets of twins, a pair of brothers and a set of three sisters. Of all the twins, only one child (case 3) was HIV infected and she shared one haplotype with her uninfected sister; the two uninfected twin pairs shared one and two haplotypes, respectively. The brothers, neither of whom were infected,

shared one haplotype. Two of the three sisters, cases 9 and 10, all of whom were HIV infected, were HLA identical. Case 8 shared one haplotye with the other two.

Discussion

The low rate of transmission seen in the Edinburgh cohort may be related to the genetic characteristics of our study population. The HLA antigen frequencies in the babies reported here, representing a relatively deprived social group, differed somewhat from our controls, which were babies of healthy parents unselected for HIV status, but who were by comparison affluent and with no history of intravenous drug use. Most notably, the relative incidence of the two commonest HLA antigen combinations (which usually occur as haplotypes) were reversed. Our data are consistent with an earlier report that A1, B8, DR3 is associated with susceptibility to infection (Steel et al 1988) and support the speculation the A3, B7, DR2 might confer some measure of protection.

On the basis of this small survey, it is clear that tissue typing would be of little value as a predictive test in babies born to mothers known to be HIV positive. There were no absolute differences in any antigenic specificity when comparing HIV infected and uninfected children, and our family studies revealed no obvious relationship between haplotype inheritance and disease susceptibility of progression. However, HLA antigens may be associated directly or indirectly with susceptibility to transplacental infection, and this might manifest itself as varying transmission rates in different populations. In

particular, a high A3, B7, DR2/A1, B8, DR3 ratio may contribute to a low transmission rate, and this possibility may merit further investigation as a specific hypothesis.

TABLE III.8.1

HLA Class 1 antigen frequencies in unrelated neonates born to HIV seropositive women

Specification	Infected % n=8	Uninfected % n=38	Total % n=46	
A1	50	23.7	28	(39)
A2	75	36.8	41	(52)
A3	12.5	42	37	(32)
A11	0	18	15	(11)
A23	0	0	0	(4.5)
A24	12.5	15.8	13	(17)
A25	0	15.8	13	(4.5)
A26	25	0	4.3	(3)
A28	12.5	2.6	4.3	(4.5)
A29	0	5.3	4.3	(5.3)
A30/31	12.5	5.3	6.5	(5.3)
A32	12.5	7.9	8.7	(3.8)
B5	0	5.3	4.3	(4.2)
B7	37.5	47	46	(31)
B8	25	18	20	(31)
B13	12.5	2.6	4.3	(5)
B14	0	10.5	8.7	(9)
B15	0	7.9	6.5	(10.6)
B16	0	5	4.3	(6.8)
B17	25	10.5	13	(8)
B18	12.5	10.5	11	(1.5)
B21	25	0	4.3	(4.5)
Bw22	0	7.9	6.5	(5.3)
B27	0	5.3	4.3	(6.8)
B35	12.5	15.8	15	(14)
B37	0	0	0	(3)
B40	0	10.5	8.7	(11.4)
B44	25	15.8	17	(27)

Figures in parentheses are the corresponding proportions for control neonates born to healthy mothers unselected for HIV status.

TABLE III.8.2

HLA class 2 antigen frequencies in unrelated neonates born to women infected with HIV

Specificity	Infected % n=7	Uninfected % n=39	total % n=46	
DR1	0	10	8.7	(12)
DR2	43	44	43	(28)
DR3	43	15	19.6	(39)
DR4	29	28	28	(25)
DR5	14	18	17	(13)
DRw6	29	18	19.6	(34)
DR7	29	33	32.6	(26.5)
DRw8	0	2.6	2	(3.8)
DRw9	0	5.1	4.3	(0)

TABLE III.8.3

HLA antigen combinations in unrelated infants born to HIV seropositive women

Combination	Infected % n=7	Uninfected % n=38	total % n=45	(n=132)
A1, B8, DR3	28.6	7.9	11.1	(24.2)
A3, B7, DR2	0	28.9	24.4	(7.6)

CHAPTER 9

INTRAVENOUS IMMUNOGLOBULIN IN HIV INFECTED CHILDREN

1) EVIDENCE FOR THE EFFICACY OF THERAPY

Aims

The aims were to evaluate the clinical effect of at least 12 months' IVIgG therapy, to document any changes in laboratory indices of viral activity, and to assess the financial implications of committing children to maintenance treatment with IVIgG.

Patients, Materials and Methods

The first eight patients identified with clinical and laboratory evidence of HIV infection were studied (cases 1,2,3,6,8,9,11,13). Clinical manifestations have been discussed in chapter 4, and detailed descriptions of the cases are found in appendix II (summarised in table III.4.1, page 111). Symptoms and signs at the commencement of IVIgG therapy summarised in table III.9.1. Monitoring and treatment regimens were as previously described.

The financial implications were calculated on the basis that the cost of immunoglobulin for a 15kg child is £45 and of materials, medical, nursing and administrative staff time, £40, estimated at 1987 prices, based on 10 minutes of medical time, 1 nurse between four

children for the duration of the infusion (2 hours), and minimal administration at each visit. This gave a total of £85 per visit, or £1600 per year. The cost of in-patient paediatric care in SE Scotland was £960 per week (CSA 1988) at the time of this study. Statistical analysis of episodes of infection and changes in weight velocity utilised the Wilcoxon rank sum test, and the days spent in hospital the Chi-squared test.

Results

After 12 months on treatment, subjective improvement in general well-being was reported in all 8 patients (see appendix II). The number of infections sufficiently severe to require hospital review was significantly reduced on therapy (fig III.9.1). Improvement in weight gain was seen in the cohort as a whole, as shown in fig III.9.2, the most dramatic change being seen in those children in whom failure to thrive had been a presenting feature. Changes in other signs and symptoms are summarised in table III.9.2.

The average number of days spent in hospital in the 12 months immediately prior to treatment (9 months from birth in case 1) was 220 (range 0-60), compared with 56 (range 0-20) in the first 12 months on IVIgG ($p<0.001$). Assuming an unchanged rate of infection, the difference between actual and expected number days of hospitalisation would be 181.6, resulting in a saving on inpatient costs of £24905, standing against treatment costs of £12800. Thus the net saving in costs is £1513/patient/year, or 49%, even without taking the travelling costs and time lost to the family in attending hospital into

consideration.

Symptomatic thrombocytopoenia, seen in 2 patients prior to therapy showed no immediate improvement on this dose of IVIgG, and a further child developed purpura while on therapy. Hypergammaglobulinaemia, seen in all patients prior to treatment, was not significantly changed by therapy, and CD4 counts continued to decline, as shown in fig III.9.3.

All patients were HIV seropositive by ELISA and virus was isolated in culture from all 8. Five children (1,2,3,8,and 11) were antigenaemic and p24Ab negative prior to the start of therapy. Changes in HIV Ag level after commencing therapy are shown in figure III.9.4, p24 Ab levels changing in a reciprocal manner. Loss of HIV Ag was sustained in three children, but case 8, who had much more advanced disease on commencing therapy, became antigenaemic again 9 months into therapy, and case 2, 30 months later (see figs III.7.1-11, page 136-146). Two children (9,13) have been consistently HIV Ag negative, and in one child (6) insufficient data are available before the start of therapy, but 12 months into treatment he was HIV Ag positive (>150pg/ml).

Treatment was well tolerated in all children. One child developed symptoms attributed to hyperviscosity, as described below, but he has had further infusions at a slower rate without incident. No other side effects have been observed.

Discussion

Anecdotal evidence of the benefits of IVIgG therapy after short periods of follow-up had been reported previously by a number of groups using regimens varying from 200mg/kg monthly (Oleske et al 1987) to 300mg/kg every 2 weeks (Calvelli & Rubinstein 1986). All demonstrated reduction in febrile episodes and number of bacterial infections. A more detailed analysis of clinical effect was provided by Schaad et al (1988), but the length of follow up was limited to only 6 months in 4 of the 7 children described. Oleske et al in a larger but non-randomised study(Oleske et al 1987) suggested that mortality is decreased. Calvelli and Rubinstein (1986) and Gupta et al (Gupta et al 1986) also noted changes in T cell and cell mediated immune function in vivo. However, in all studies, interpretation of results was hampered by the small numbers involved and the lack of control data. During the course of our study, a large multicentre trial was commenced in the United States (NICH IVIgG Study Group 1991). Because of the limited treatment options, and the small number of children involved, at the time when this study was carried out, we felt an untreated control group was not appropriate. However, because we followed these children prospectively from the time of their diagnosis as being HIV positive, we obtained reliable sequential data for a comparable 12 month period immediately before treatment was commenced. This enabled us to use each patient prior to treatment as his own control. Long term studies now suggest that in children surviving past the first year of life, the symptoms of HIV children tend to improve in the second year(ECS 1991). The age range of the children we studied was very wide (9 months to 47 months) so although it is possible for spontaneous improvement to have occurred in cases 1

and 3, it would have been less likely for the others. In addition , no previous group had documented changes in HIV Ag and p24 Ab levels following treatment, nor had the financial implications been assessed.

Our group showed a great deal of heterogeneity in terms of age, duration and severity of symptoms prior to therapy, posing difficulties when analysing the cohort as a whole. These were partly overcome by using the patient as his own control, and also, for example, by standardising growth data in terms of velocities. The choice of our dosage regimen aiming to provide the optimum benefit with minimum distress for the child was based upon data from treatment of primary hypogammaglobulinaemia (Leen et al 1986). It is possible, however, that higher doses may be required more frequently in hypergammaglobulinaemic patients, since the half life of IgG is reduced at higher serum concentrations. This issue of optimal dosage will only be resolved by further studies of efficacy.

Disappearance of HIV Ag has been described in adults following the infusion of plasma high in p24 Ab and neutralising capacity has been described previously (Jackson et al 1988). No studies of normal immunoglobulin therapy have documented changes in HIV Ag, and in view of the small numbers involved, further studies will be required to verify these findings. The mechanism of action is likely to be different, as plasma donors are screened for HIV infection and the IVIgG preparation has no anti-HIV antibody. The production of HIV Ag is an indicator of enhanced expression of viral genes (Nabel 1988), and may reflect the expression of latent virus following activation of helper T cells. Bacterial and viral infection increase T cell activation, and the

disappearance of HIV Ag following therapy may be due to the reduction of viral activation following the decrease in infective episodes. The continued decline in the CD4 count was disappointing, but the disappearance of HIV Ag with IVIgG therapy suggests that the progression to AIDS may be delayed, since the loss of p24 Ab and the appearance of HIV Ag are recognised bad prognostic signs(Lange et al 1986).

The clinical benefit of therapy we have demonstrated, together with the quality of life enjoyed by our patients leads us to conclude, with Schaad's group (Shaad et al 1988), that there is no case for withholding therapy in symptomatic children, pending the results of the double blind placebo controlled trial(NICH IVIgG Study Group 1991). What remains unclear is at what point in the course of disease treatment should be commenced, given that 5 of our children were in the early stages of symptomatic disease (CDC stage P2 A/F). Further studies are also needed to optimise the dosage regimen. A controlled trial will be necessary if we are to consider treatment in asymptomatic children. This is likely to be problematical because of the lack of a reliable diagnostic test of HIV infection in young children. As the number of HIV infected children grows over the next few years, routine therapy has major implications in budgeting of hospital in patient and day care facilities, which we have addressed by demonstrating the cost benefit of therapy, at least in the short and medium term. It is unlikely, however, that IVIgG will delay progression indefinitely, and further advances in immunotherapy, such as the introduction of specific anti-HIV neutralising antibody in combination with IVIgG and anti-retroviral therapy will be required if we are to improve the ultimate prognosis for

these children.

2) HYPERVISCOSITY AS A POTENTIAL HAZARD DURING THERAPY

Aims

To report an HIV infected child in whom infusion of IVIgG led to serious symptoms suggestive of hyperviscosity.

CASE REPORT

The patient (Case 13) became infected with HIV after blood transfusion at the age of 4 months, before screening of blood for HIV antibody was available. His history is summarised in appendix II. At 40 months he was commenced on regular infusions of IVIgG according to the standard regimen.

Treatment with IVIgG was not associated with any adverse reactions until the age of 4 years 10 months, when, due to an infusion pump malfunction, the patient received the infusion 30% faster than usual. He suddenly became confused, distressed, and was unable to see. He was dysarthric before becoming totally aphasic, and a transient right facial palsy was noted. Pupils remained equal and reactive to light, eye movements remained full and no abnormality could be detected on ophthalmoscopy. Tone, power and reflexes remained equal and symmetrical. The IVIgG was immediately discontinued, and a normal saline infusion set up. After 30 minutes, he

fell asleep. Two and a half hours later there were no residual neurological symptoms or signs. In retrospect, his parents commented that he had had very brief episodes of confusion and drowsiness during previous IVIgG infusions, on one occasion waking up after a sleep appearing unable to speak. The effect had lasted a few minutes.

On the day of his severe adverse reaction his serum IgG, IgA and IgM levels were 53.2g/l, 1.3g/l and 4.8g/l respectively. No paraprotein bands were found on protein electrophoresis. His serum relative viscosity measured by capillary viscometer was 5.0 before and after the infusion (normal range 0.42-2.78). A computerised tomography scan subsequently showed no abnormality, and the patient has had no recurrence of symptoms suggestive of hyperviscosity, continuing his 3 weekly infusions at a slower rate of 1.3ml/kg/hr. Subsequent viscosity measurements have varied between 3.0 and 5.0 (data not shown) and there was no difference in viscosity levels before and after IVIgG therapy. Immune complexes were also measured before and after IVIgG therapy and no change was observed.

Following this incident, we studied a further 7 children (Cases 1-3,6-9) treated with IVIgG, none of whom had suffered side-effects associated with IVIgG infusions. We also investigated 20 of the children born to HIV seropositive mothers who were presumed uninfected. The mean ($\pm$ SD) viscosity in the HIV infected group was significantly raised with a value of 3.2 $\pm$ 0.96) compared with the non-HIV infected group (2.3 $\pm$ 0.39; $p<0.01$, Student's t- test).

The relationship between serum IgG, IgA and IgM levels and the serum relative viscosity was investigated in the above samples and additional samples collected from the HIV infected children and the serum relative viscosity was found to correlate both with IgG levels ($r=0.74$, $p<0.001$) and with IgM levels ($r=0.66$, $p<0.001$). There was no significant correlation between serum viscosity and IgA levels (Table III.9.3). In other HIV infected children, serum viscosity was measured on stored samples taken before and immediately following IV IgG infusions. No rise in viscosity could be demonstrated in any sample. Protein electrophoresis revealed no oligoclonal or monoclonal bands in any of the specimens tested.

Discussion

Intravenous immunoglobulin has been used widely for the treatment of primary hypogammaglobulinaemia and ITP. Adverse reactions are rare, and in most clinical situations, serum immunoglobulin levels are low or normal in the recipients. In HIV infection, serum IgG levels prior to IVIgG infusion are usually high, and problems with hyperviscosity had only once been previously described in an HIV infected adult (Martin et al 1989).

The hyperviscosity syndrome has been described in adults with paraproteinaemias (Fahey et al 1965), and in patients with autoimmune and rheumatic diseases, when it is attributed to aggregates of intermediate size, or to polyclonal IgG polymers (Somer et al 1980). Although we have shown that the serum viscosity is raised along with serum immunoglobulin levels in these children, there have been no previous

reports of hyperviscosity symptoms occurring after IVIgG infusion. Clinical manifestations are rare in patients with viscosities less than 4 (Somer 1987). However, the 'symptomatic threshold' may be very variable, although remaining constant for any given patient. In case 13, the threshold was exceeded during one of the IVIgG infusions; no other patient developed symptoms during a rapid infusion of IVIgG.

CNS involvement in HIV infection is discussed in chapter 4. We could not definitely exclude CNS infection in our patient as we did not have the opportunity to examine his cerebrospinal fluid at this time. Nonetheless, it is possible that in a HIV infected patient with very high IgG levels, hyperviscosity might contribute to symptoms previously attributed to CNS infections and that methods of treatment which lower viscosity may be beneficial.

On the basis of our findings, we therefore recommend that in HIV infected children with very high serum immunoglobulin levels, the clinician is alerted to the problem of hyperviscosity. Caution should be exercised in these circumstances, and a slow rate of infusion of IVIgG chosen.

Table III.9.1.

Clinical features of the children on commencement of IVIgG

Case	Age 1st symptoms (mths)	IVIgG started (mths)	CDC Stage	Clinical features
1	3	9	2A	Recurrent URT sepsis Diarrhoea Eczema Failure to thrive
2	9	24	2A	Recurrent URT sepsis Recurrent pneumonia Diarrhoea
3	9	14	2A	Recurrent URT sepsis Recurrent fever Eczema
6	12	37	2F	Recurrent URT sepsis Night sweats Purpura Eczema
8	9	47	2D2D3	Recurrent pneumonia Diarrhoea Oesophageal candida Failure to thrive
9	18	24	2C	Recurrent URT sepsis Recurrent pneumonia LIP Eczema
11	22	26	2B	Prolonged pertussis Recurrent pneumonia Encephalopathy Diarrhoea Failure to thrive
13	14	42	2C	Purpura Pneumonia Oral candida LIP

Table III.9.2

Symptoms and signs of the children before and in the 12 months after commencing IVIgG

Feature	Before	After	p
Total (median) no. episodes pneumonia	13(2)	4(0)	NS
Total (median) months diarrhoea	49(9)	21(3)	<0.05
No. patients with: URT sepsis	8	8	NS
Eczema	5	5	NS
Lymphadenopathy	8	6	NS
Hepatosplenomegaly	7	8	NS
Thrombocytopoenia	2	2	NS

Table III.9.3.

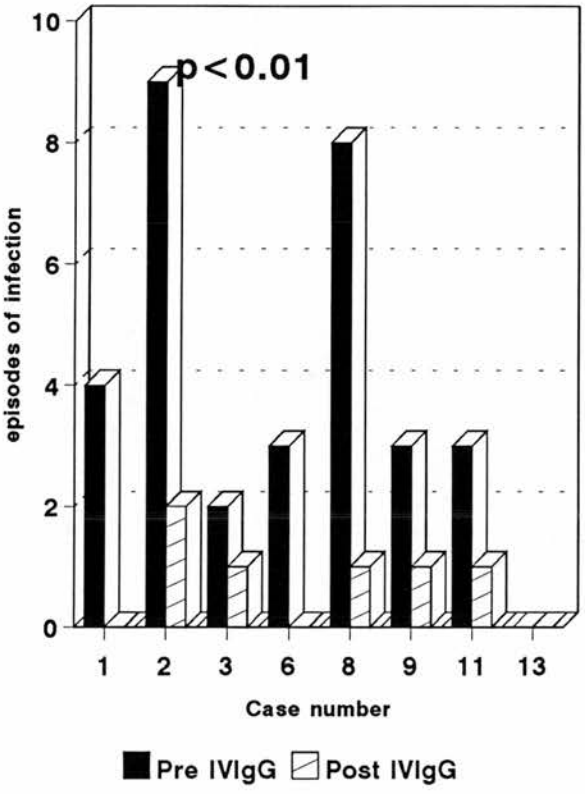
Serum Viscosity and Immunoglobulin Levels of HIV Infected Children.

Case	CDC Stage	IgG	IgA	IgM	Viscosity
13	P2C	53.2	1.3	4.8	5.0
9	P2C	46.8	0.3	2.3	3.5
6	P2F	29.6	3.3	2.4	3.5
3	P2A	24.0	0.7	2.2	2.0
7	P2A	18.8	0.7	1.2	3.6
2	P2A	17.2	1.2	0.9	3.2
1	P2A	14.8	1.6	1.1	3.0
8	P2D3	14.4	2.2	1.3	2.0

FIGURES III.9.1-4

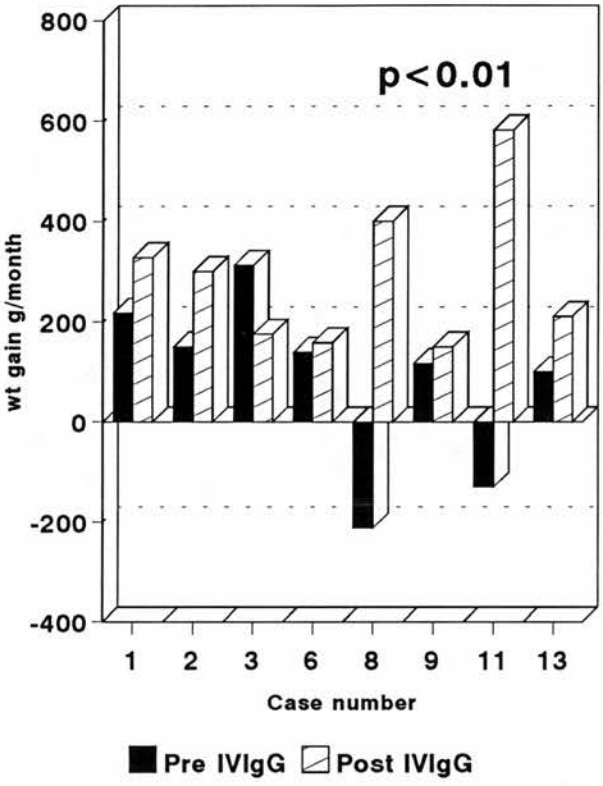
1

Number of episodes of infection



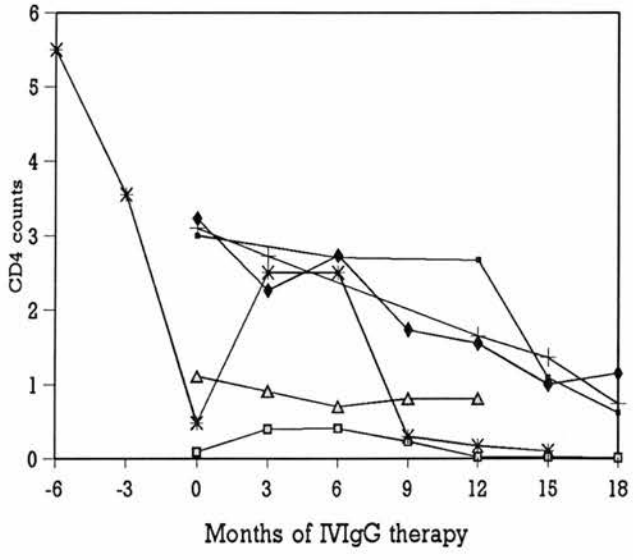
2

Weight gain



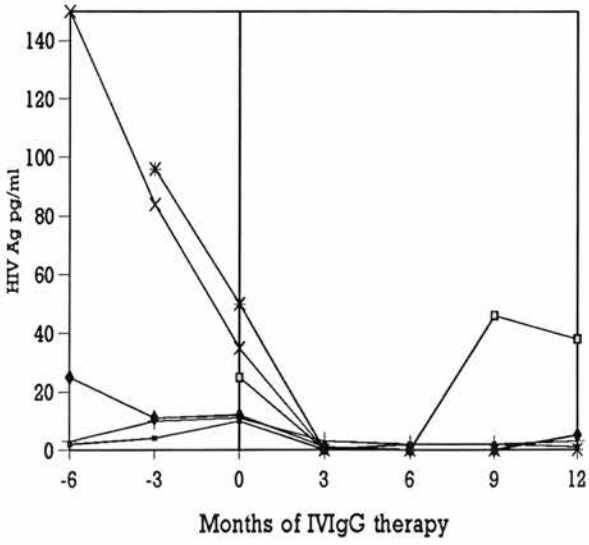
3

Change in CD4 counts after IVIgG



4

Change in HIV Ag after IVIgG



CHAPTER 10

THE USE OF ZIDOVUDINE IN SYMPTOMATIC HIV INFECTED CHILDREN

Aims

To study the clinical effects and toxicity of zidovudine in children with symptomatic HIV infection.

Patients and methods

Cases 1,2,6,8,11, and 13 received zidovudine for a median of 25 months (range 12-42). All had already received IVIgG for a median of 18 (range 1-31) months previously. Cases 8 and 11 had received IVIgG for 2 and 1 months only before commencing zidovudine. Clinical features are detailed in chapter 4 and appendix II and the laboratory parameters in chapter 7. The principles of dosage and administration are outlined in section II. Details for each case are summarised in table III.10.1.

Results

Clinical benefit was seen in terms of improvement in neurological signs and symptoms in both patients with evidence of encephalopathy at the time when zidovudine was introduced, most dramatically seen in case 11. Case 8 who had been on IVIgG for 2

months had no recurrence of oesophageal candidiasis once zidovudine was commenced, and also suffered fewer infections. The other 3 children had mild non-specific symptoms and signs only on commencement of therapy, and although they remained stable, showed no dramatic improvement. Of the four children who commenced zidovudine more than a year after IVIgG only one had been admitted to hospital because of infection on one occasion in the previous 12 months, and so no change in rate of infectious episodes could be demonstrated. Weight gain in the 12 months before and after AZT was commenced is summarised in fig III.10.1; gain in weight for each case is illustrated in figs III.7.1-11, page 136-146, and similar data for case 13 in fig III.10.4. There was a significant improvement in weight gain ($p<0.01$, Wilcoxon rank sum) for the group as a whole, the 4 children who were least well at the start of therapy showing the greatest change.

Five children had a history of HIV antigenaemia. No clear relationship was observed between commencement of therapy and change in HIV Ag or p24 Ab (fig III.10.2). HIV Ag reemerged during therapy in cases 2, 6, and 8 after 1-14 months, perhaps representing zidovudine resistance. CD4 counts were low in all children prior to commencement of therapy, median $0.44 \times 10^9/l$, range $0.05\text{-}0.64 \times 10^9/l$. Following AZT, levels stabilised or rose in the next 6 months before declining again at a slower rate than previously ($p<0.05$, Wilcoxon signed rank test, fig III.10.3).

Compliance with therapy was good on a TID regimen in all but one patient (case 8), despite all being started on the intravenous

preparation. This had a foul metallic taste, and had to be disguised in juice. Zidovudine in case 8 was administered via a nasogastric tube because of her refusal to swallow it, and her compliance was erratic. This was reflected in MCVs, which in the other 5 rose from a mean of 78 (range 68-85) to 103 (range 96-114) over the first year, whereas in case 8 they remained unchanged (85 to 87).

Early side effects were seen in case 2, who developed headaches and aching limbs between 2 and 4 weeks into therapy. No action was taken and symptoms subsided spontaneously. Two children (cases 8 and 11) developed marrow toxicity 6 and 9 months into therapy, both requiring blood transfusions for low haemoglobin. Unlike the other children, both these cases had had low haemoglobin levels before treatment was commenced. In case 8, AZT was stopped when a neutropoenia developed ($0.8x10^9/l$). A pancytopoenia developed in case 11, haemoglobin falling to 7g/dl, total white count to $0.8x10^9/l$, and platelets to $42x10^{12}/l$. AZT was stopped for 2 weeks and then recommenced at half dosage. Zidovudine was discontinued in case 6 because of marrow depression during chemotherapy, but no toxic effect had been seen prior to this. In the others, haematological parameters (excluding MCV) remained stable. Mean platelet count was $199x10^{12}/l$ on commencing AZT, and $212x10^{12}/l$ after one year, a difference which is not significant, and no transient rise was seen during the first months.

Discussion

By the time the children in our series were commenced on

zidovudine, information from studies in large groups of adults, and results of preliminary studies in children demonstrating its clinical benefit in delaying disease progression were already available. As the number of symptomatic children available for study in our series was small, no controlled trial was considered either possible or appropriate. Cases 1, 2, 6, and 13 had been receiving IVIgG for at least 17 months before AZT was commenced. However, cases 8 and 11 commenced AZT only shortly after IVIgG, so the individual contribution of each therapy to any change in condition is impossible to distinguish. Our findings are compatible with those of the recent large multicentre trial of oral zidovudine, (McKinney et al 1991), which has demonstrated a marked improvement in cognitive function in those under 3 years, and in weight gain on a dose of 720mg/m^2/day in 4 divided doses. Results of long term follow up in this study are not yet available, but our experience suggests that these benefits demonstrated initially are not maintained, and further progression of HIV disease is not delayed indefinitely.

Our patients all commenced oral zidovudine when HIV Ag levels were low or negative. We were therefore unable to confirm previous observations of the reduction in HIV Ag following therapy (Epstein et al 1988 [b], Blanche et al 1988, Jackson et al 1987). However, despite a raised MCV, suggesting adequate compliance and absorption, HIV Ag became positive in two children and persisted while on therapy, and reappeared transiently in a further two. Such gradual reemergence of HIV Ag has also been described in adults (IG Williams et al 1990). This suggests that viral suppression may be incomplete or the development of resistance to zidovudine. We were unable to demonstrate any

consistent increase in p24 Ab following therapy (Blanche et al 1988).

A rise in CD4 count following zidovudine therapy is well recognised (Dournon et al 1988, Fischl et al 1989, Blanche et al 1988, McKinney et al 1991), but after reaching a peak at around 2 months, counts decline to pre-treatment levels by 4 to 5 months, and continue to decline thereafter. The variation in response to zidovudine in our group of patients may reflect variation in the time taken to develop resistance, but it is encouraging that CD4 counts remained stable in cases 1 and 2 during 3 years on therapy.

Three children remained free from side effects, despite prolonged use of the drug. McKinney et al (1991) also found AZT to be well tolerated, but side effects included vomiting (5%), insomnia (3%) nervousness (2%) and abdominal pain (2%). Haematological toxicity occurred in 61%, with 26% suffering from anaemia and 48% neutropoenia. 23/88 children required transfusion. Clinical findings suggestive of encephalopathy on dose reduction, such as occurred in case 6 has been reported previously(Helbert et al 1987). There are, however, no other reports in children. Overdosage has been described, with no adverse effect (Moore et al 1990). This is an important consideration, given that the drug has to be given in households many of which are at greater than average risk of accidental poisoning within the home (Sibert 1975).

In adults on long term therapy, adverse reactions have decreased over time (Fischl et al 1989), but concerns over long term toxicity remain. Myopathies have long been described(Bessen et al 1988, Gorard et al

1988), and the mitochondrial myopathy reported more recently may occur after an average of 12.8 months of therapy (Dalakas et al 1990). ECG changes were seen in 3% of children in McKinney's series, and echocardiographic abnormalities in 2% (McKinney et al 1991). It is concerning that two children in our series developed cardiomyopathy on therapy. Case 13 had a history of major cardiac surgery, but he had normal contractility until he had been on zidovudine for 18 months, and case 2 died of cardiac failure associated with a dilated cardiomyopathy. Therefore the possibility of this being an AZT-related phenomenon must be taken seriously. It is disappointing that no autopsy material was available from case 2 for study.

Our findings are therefore in keeping with others' (Pizzo et al 1988 [b], Blanche et al 1988, McKinney et al 1991) that in children with symptomatic HIV infection, the benefits of treatment outweigh the risk of toxicity. Further work needs to be done to determine the optimal dosage regimen to achieve maximum antiviral activity with minimal toxicity, and to determine the optimal time to commence therapy.

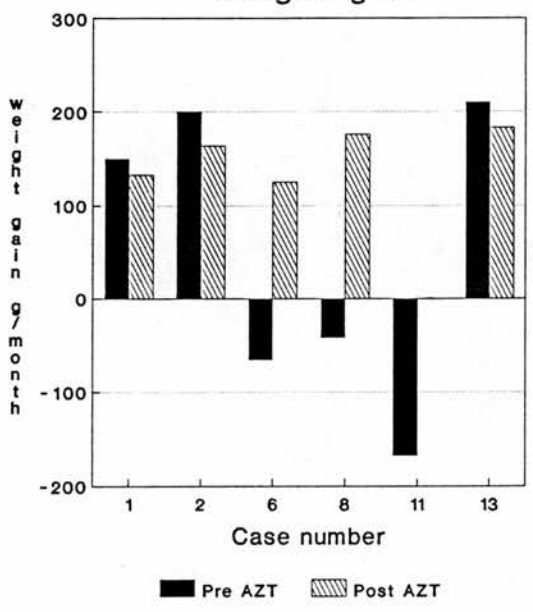
TABLE III.10.1

Clinical features of children on commencement of zidovudine

Case	AZT started (months)	Dose (months)	CDC stage	Clinical features
1	28	14mg/kg/day (20) 400mg/m^2/day (21)	P2A	History of URT sepsis Falling CD4 count
2	42	14mg/kg/day (14) 600mg/m^2/day (23)	P2A	Widespread molluscum contagiosum Falling CD4 count
6	58	14mg/kg/day (12) 600mg/m^2/day (7)	P2C	Thrombocytopoenia LIP Intermittent parotitis Failure to thrive Falling CD4 count
8	49	400mg/m^2/day(3+7)	P2D1,2	Recurrent pneumonia Diarrhoea Oesophageal candida Failure to thrive
11	28	400mg/m^2/day (10)	P2BD3	Encephalopathy Prolonged pertussis Recurrent pneumonia Failure to thrive
13	63	14mg/kg/day (21) 600mg/m^2/day (10)	P2C	LIP Falling CD4 count CNS symptoms

FIGURES III.10.1-3

1



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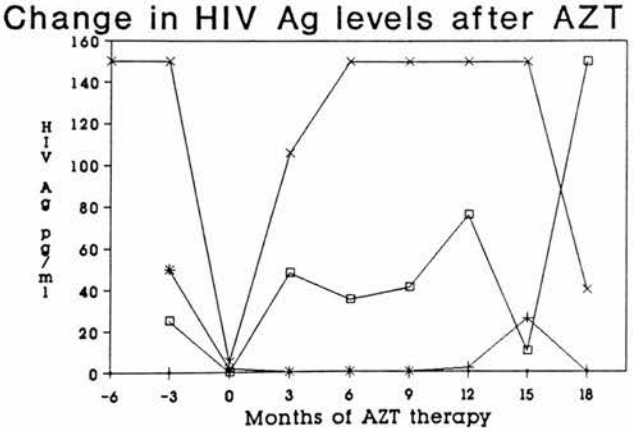

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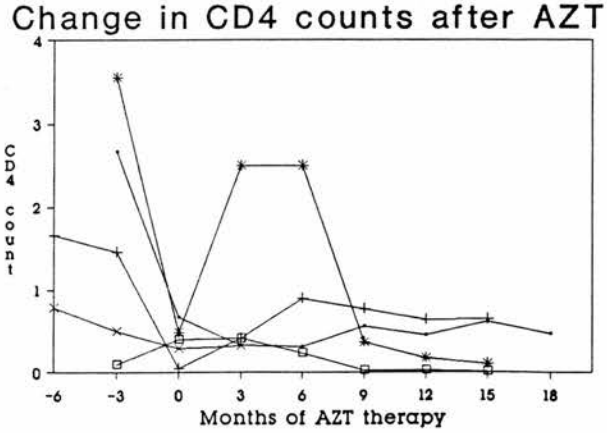
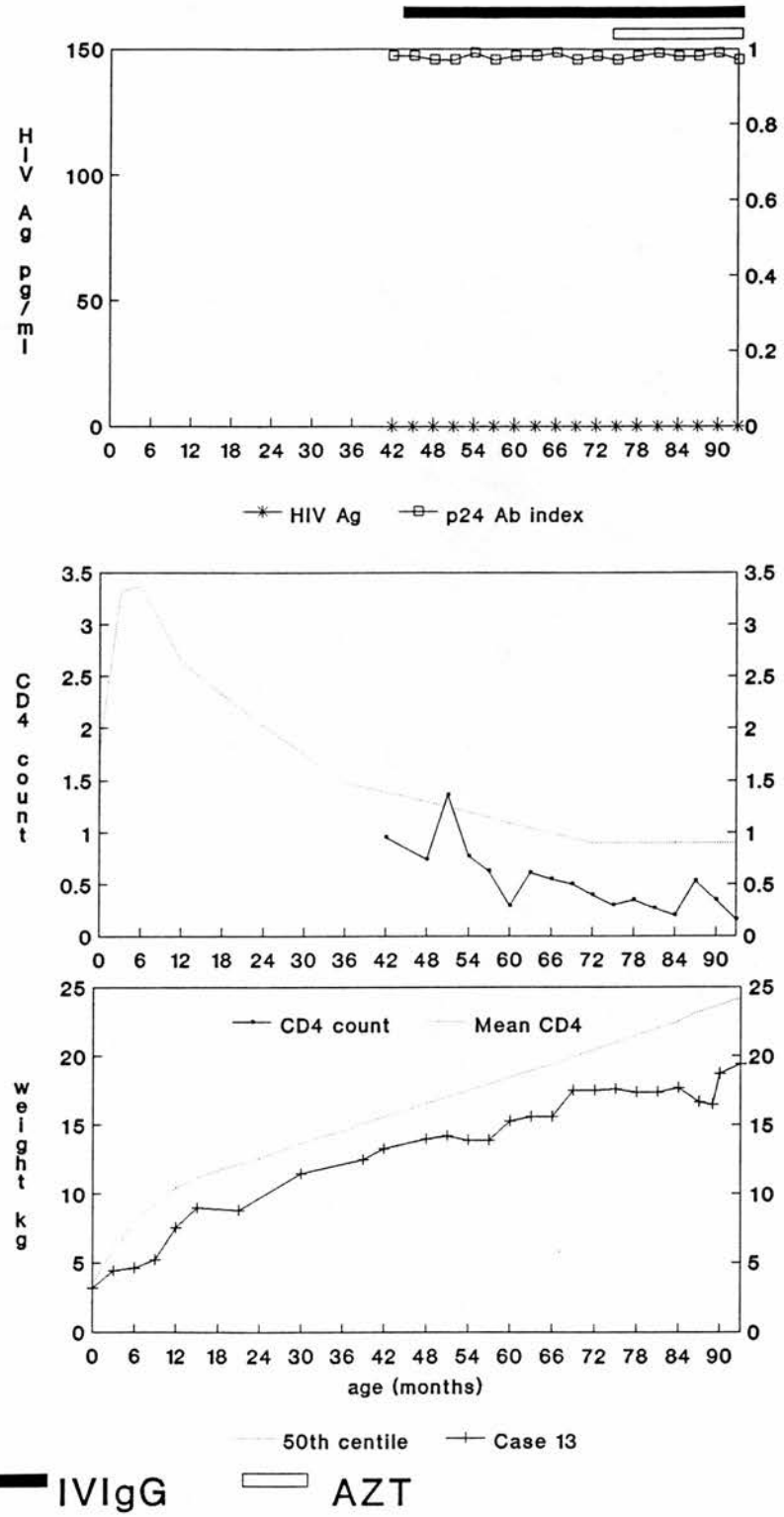


FIGURE III.10.4

Laboratory parameters for case 13



CHAPTER 11

MANAGEMENT OF CHILDREN BORN TO HIV SEROPOSITIVE WOMEN - A COMPARATIVE STUDY

Aims

Although numbers of children at risk of HIV infection are growing, experience in any one centre at this time is limited, and so collaboration between centres is essential if the maximum information is to be obtained in the shortest space of time. This has been achieved in the European Collaborative Study, which involves 10 centres in 6 countries (ECS 1988). Standardisation of procedure and data collection is therefore required, but the practicalities of obtaining this data may be extremely variable. Factors influencing management will include cultural (or sub-cultural) differences, socio- economic background of families involved and their compliance. Other factors include levels of medical and technical staffing and the expertise of individual members of the team, as well as the physical lay-out of the clinic and the accepted methods of medical practice within an institution.

My aims in this study (sponsored by the Council of Europe) were therefore to compare the practical management of children born to HIV seropositive mothers in a Southern European centre (Padova, Italy), and in a Northern European Centre (Berlin), with that of our own centre in Edinburgh. I also aimed to increase my experience of the

spectrum of manifestations of HIV disease in children, and of therapy.

Methods

In September 1989, I visited Universita degli Studi di Padova, Italy, and Universitatklinikum Rudolf Virchow, Freie Universitat, Berlin (before the reunification of Germany) for two weeks each. I attended the HIV clinics, interviewed staff, reviewed the patient's case files, and in Padova, spent some time in the virology laboratories.

The Setting

The social geography of the three centres is compared in table III.11.1. Padova is the regional centre of referral for paediatrics from the province of Veneto. The city itself is small, population 230,000, and many of the patients live in the surrounding small villages and towns. This meant they had to travel considerable distances to attend the clinic. Although in 95% of the children seen, there was a history of drug misuse in at least one parent (in 10% the father only). Unlike Edinburgh, very few of the mothers appeared to knew each other from any drug sub-culture. Practically all had adequate housing and a telephone, and the majority drove cars. By contrast, Berlin is a huge city, population 2 million. Because of the difficulties of travelling to the rest of West Germany, it was a static population. There were chronic housing shortages, and many young people wanting to leave the family home resorted to 'squats' or living in caravans. It is in such areas that drug use occurred and from which many of the children studied came.

In Italy, the cohesion of the families affected by HIV remained strong. Many mothers were married or in stable relationships and much of the support for the mothers came from the extended family. This was also reflected in the small number of children requiring fostering or adoption. Children were usually cared for within the family until school age, and so it was when children at risk of HIV infection were reaching five or six years, that the problem of integration into a peer group had to be addressed. In Berlin and Edinburgh, many women were not living in stable relationships, and their lifestyle were less compatible with good child rearing. In Berlin, children have required foster care, but none as yet has been adopted. Although day care, and kindergartens were well established in Berlin, priority for places went to mothers who had employment outside the home. If women received benefit from the state to live, it was considered that there is no reason why they should not care for their children, and so they tended to come lowest in the list of priorities. Some children had been found places, however. Both in Berlin and Edinburgh many of the issues relating to the care of children at risk of HIV infection within such institutions had been addressed.

The clinics and surveillance procedure

All children were seen according to the protocol of the European Collaborative Study, although only those children identified from birth qualified for inclusion into the study itself. The system and personnel used for the surveillance varied greatly, as summarised in

table III.11.2. In both centres I visited, some HIV infected children from risk groups other than vertical transmission were followed, whose data I have not included.

In Padova, all routine reviews took place in the once weekly, clinic, where up to 10 children were seen in a morning. Infants were delivered in and referred from maternity hospitals covering a wide area, so few were known to the clinic antenatally. Cord blood might be collected by the staff in the maternity hospital at delivery, but this did not occur in majority of cases. The first contact with the clinic was therefore at a month or 6 weeks of age.

The general impression was one of a very formal setting. Different rooms were occupied by the nurses, the paediatrician, the neurologist, the neuropsychologist, and the adult physician. On arrival, the child's blood was taken. Then followed the general medical interview and examination, which could involve the participation of up to three medical personnel. The child then received a detailed neurological examination, again in accordance with a strict protocol, and then a neurodevelopmental assessment. All infected children had a yearly CT scan and CSF was obtained while they were under sedation. At some stage, if she wished, the mother could also be seen herself. The whole process took most of the morning, making it a long day for those having to travel some distance. Despite this, the children were on the whole cooperative, reflecting the attitude of their parents who seemed to recognise the importance of the child's medical review. However, as attendance at the clinic depended solely on the motivation of the parents, it may be that those who were less motivated

were the ones who failed to attend and were lost to follow-up. There was no system of home visiting.

In Berlin a small set of rooms were designated for use by the HIV clinic. Up to four children were seen every day for routine follow-up or treatment, but rarely would more than one or two be on the premises at the same time. One doctor would conduct the interview with the help of the nurse, and carried out the general and neurological examination, and venepunctures. The whole visit lasted on average, about an hour. The older children were assessed by the psychologist on a separate occasion. The social worker was available so that relevant problems could be discussed and help sought during the same visit. Compliance was high when the children were in alternative care, and in all cases was greatly enhanced by the taxi service arranged for and paid by the hospital to bring the families for their appointments. Three home visitors deal with practical help for the families, such as housework and child care for mothers who were unwell, as well as nursing procedures at home.

Laboratory Evaluation

All centres performed a core of laboratory tests as agreed by the European Collaborative Group, and listed in section II. In addition, biochemical parameters, including urea and electrolytes, liver function tests and proteins were measured in Padova and Berlin. In Berlin protein electrophoresis was performed and IgG subclasses measured. The virological tests available at each centre are listed in table III.11.3.

Results

Tables III.11.4 and III.11.5 give details of the children being followed in the 3 centres, and their clinical features. The experience of the natural history of the disease is similar in all 3 centres, the onset of symptoms occurring in the first year for the majority of children. In Berlin and Edinburgh there were deaths not necessarily associated with HIV, Berlin having a sudden infant death in a five month old.

In all three centres, all symptomatic children remained HIV seropositive at each test. Table III.11.6 shows the results of other laboratory tests. In Padova, in vitro antibody production in the first months of life was a useful indicator of infection in 8 of the 9 infected children followed from birth.

The diagnosis of HIV infection in children who were asymptomatic, or with symptoms not specific for HIV posed problems. In Padova 5 children classed as stage P1 had other immunological abnormalities supporting the diagnosis, as had 2 in Berlin. All eight children were hypergammaglobulinaemic, 7 had CD4/CD8 < 1.0, but only 2 had an absolute CD4 count $< 1.0 \times 10^9/l$. Seven had persistent HIV antibody beyond 18 months, the other child seroreverted and remained negative. Seven were virus culture positive on more than one occasion, and 7 had been HIV antigenaemic on more than one occasion. Of the 5 Padova children, all four who were tested were positive for IVAP. Although none had symptoms definitely related to HIV, 7 of the 8 had persistent lymphadenopathy, 4 had

hepatosplenomegaly, one failed to thrive, one had recurrent diarrhoea, and one recurrent fever.

There was another group of children for whom the basis of HIV infection is made on virological parameters alone. In Padova, 6 children were defined as stage P1A. Four were over 18 months and had seroreverted to become HIV antibody negative. Five were virus isolation positive on one occasion, and 1 on 2 occasions. Three were positive by PCR on more than 2 occasions, 2 on one occasion, and for one the result was equivocal. Two had been HIV antigenaemic, and 2 produced a response on IVAP. For two children, the diagnosis of HIV infection was based on viral culture alone, but the other four had had a combination of positive tests.

In Berlin, although many children had at one time one positive culture or viral antigen result, in only 5 children was this found on more than one occasion, or by more than one test. A further child had no virological indices of infection, but persistently low CD4 counts, and decreased reaction to mitogen stimulation, suggestive of impaired cell mediated immunity.

Treatment

Options available for the treatment of symptomatic HIV infection include specific treatment for infective episodes, IVIgG infusions and zidovudine. The criteria for the use of IVIgG in Edinburgh is described in chapter 9, and children were treated in the same paediatric ward to which they were admitted during any acute illness. Similar criteria were

used in the other centres. Twelve children in Padova, including the 4 who died, were treated monthly with a dose of 400mg/kg. One room in the clinic is set aside for this purpose, so that the children were assessed during the weekly clinic. In Berlin 8 children were treated monthly, also with 400mg/kg. Most children received their treatment in the clinic, but they sometimes went to a paediatric ward if they were well known there.

No definite criteria for commencing zidovudine had been established. Five children meeting the criteria for paediatric AIDS were being treated in Padova, and 2 in Berlin. Until recently all were using the regimen of 400mg/m^2/day. Padova used a twice daily dosage regimen, Berlin four times daily. On this small sample, no conclusion could be drawn on clinical benefit, and no pattern could be seen in laboratory parameters before and after commencing therapy. One child in Berlin had suffered myelosuppressive side effects, with anaemia and neutropoenia. In Padova and Edinburgh, children on zidovudine were reviewed at the time of their IVIgG infusions, which they all received, every 4 and 3 weeks respectively. In Berlin, blood counts were checked every 2 weeks because of the danger of myelosuppression, and the neutropoenic child was seen weekly.

CMV retinitis is a complication I had not seen before my visit to Berlin. In this child, its progression was extremely rapid, affecting both eyes to render the child almost completely blind in the space of two weeks. The child was being treated with CMV hyperimmune globulin, which because of its short half life in vivo, has to be given every 2 weeks. Another child in the Berlin group without CMV related

symptoms was also being treated on this regimen after being found to excrete CMV chronically from the nasopharynx and in urine. She received regular ophthalmology checks.

Discussion

My visit to two other European centres gave me the opportunity to compare and contrast different methods of management of children with the same spectrum of clinical problems, and where the same research questions were being asked. Provision of the optimum level of care means that a balance has to be struck between what is ideal for the child, what is acceptable to the child and its family, and the requirements of the research, which is also in the patient's interest. Constraints include available resources, both financial and in terms of the background and training of the personnel involved.

The differences between centres in assumption of roles is illustrated well by that of the nurse. In Padova, the nurse was primarily the technician, who carried out the practical procedures such as venepuncture and setting up the infusions, and was neither involved in management decisions, nor in counselling. In Berlin, she was the first point of contact when the child came into the clinic, and her role was that of assisting the doctor with practical procedures, and she carried the major part of the administration. In Edinburgh, a specialist nurse, the health visitor, with additional skills in screening of child development, and in health surveillance within the community. assisted the doctor during visits, but was not involved in the administration of the project, and did not perform technical procedures.

In Italy, the importance given to the family in society, and so to the children within it, meant that most parents appreciated the value in having their children checked even if they appeared well to reassure them this was the case. They were sufficiently motivated to travel long distances at their own expense, and to spend a long morning at the hospital for this to be achieved. The advice given by the physicians at the visit was generally accepted and complied with. The chief role of the clinic was seen to be the provision of medical care and the generation of research data.

Both in Berlin and in Edinburgh, the social circumstances of the families were much more prominent in dictating the management of the children. HIV infection was only one factor influencing the morbidity in these families, and addressing other issues may be equally important for the overall well-being of the child. Thus part of the role of the clinic was to facilitate the provision of care in other areas than purely medical. This involved liaison with the psychologist (for mother's needs), and with other agencies such as fostering agencies, and those involved in day care and education. These other services provided by the clinic were important motivation factors for the family, particularly if the child is clinically well.

The long term nature of follow up necessitated continuity and consistency in care. When only a small number of children are involved, a single doctor can provide this. However as numbers grow this ideal becomes increasingly impractical. In Edinburgh, during the period of study, this continuity was provided by only 2 doctors working

in close cooperation, one of whom met the mother and child at birth, which is a good time to develop a good rapport. In Berlin, the number of medical staff involved was still small enough to allow rapport to be developed, even though staff may eventually change, and regular communication with the coordinating physician ensured continuity. The large number of personnel involved in Padova meant that consistency has to be achieved by adherence to a rigid protocol. A meeting of all the personnel following the clinic to discuss any problems assisted continuity.

HIV is a family disease, and paediatric AIDS cannot be viewed in isolation. The ability of a mother to cope will be diminished if her health or that of her partner deteriorates. In both centres I visited, I was told that good liaison existed with the adult physicians, but in Berlin, this did not extend to the detailed exchange of clinical data. The health of the mother was well known in as much as it affected her ability to care for the child, and the home visitors were involved in the care of the family as a whole on this basis. In Padova, some data from mothers was collected in the paediatric clinic, but data on the mothers prior to delivery was less readily obtainable.

In this area particularly, the advantage of the paediatric follow up being based alongside the adult clinic, as in Edinburgh, was clearly visible. Not only could data be exchanged freely, but information about other family members could also be gleaned informally, and knowledge of women in the clinic who were found to be pregnant enabled forward planning and preparation. Joint clinical meetings with the staff in the adult clinic also gave a broader base of knowledge of

the local progression of the HIV epidemic and put the developments in the children into perspective. The main disadvantage was that although the premises were used for infectious diseases clinics other than HIV, few other children were seen, and some mothers were very conscious that if their children were seen in the clinic, others would presume they were HIV infected. This problem was partly combated by having the option of home visiting.

Cooperation and communication are essential to the advancement of knowledge of HIV, and multicentre studies will achieve results in a shorter time than would be possible in one centre alone. However, it is important when devising trial protocols or guidelines for management to take into account cultural factors which will influence their effectiveness. Even between different European cities it is inadvisable to take a regimen of management which may work excellently in one place, and copy it absolutely in another. Similarly, the same protocol may be executed in very different ways in different centres, and this may influence the results obtained. It is even more important to recognise this when devising protocols for use in situations as diverse as the USA and central Africa, urban ghettos and rural communities, among drug misusers and those from areas of chiefly heterosexual transmission.

TABLE III.11.1

Demographical features of the 3 centres

	Padua	Berlin	Edinburgh
Population	230,000	2,000,000	550,000
Area of referral	Large district	Within city	Majority within city
Major risk factor for HIV	Drug use	Drug use	Drug use
Deprivation	No	Yes	Yes
Family support	Good	Poor	Variable-often poor
Fostered/adopted	<5%	33%	10%
Pre-school day care	Unusual	Difficult to get	Common

TABLE III.11.2

Details of the clinics

	Padua	Berlin	Edinburgh
Staff	Paed (ID) Neurologist Psychologist 7 junior paeds Nurse	Paed (oncology) Neurologist Psychologist 2 junior paeds Nurse Social worker Home visitors	Paed (community) Health visitor Paed research fellow Dental hygienist Social worker Volunteer help
Time of first interview	6 weeks	4-6 weeks	Antenatal
Designated clinic space	No	Yes	Yes
Site most children seen	Clinic	Clinic	Home
Medical examination	Paed	Paed	Paed
Venepuncture	Nurse	Paed	Paed
System of developmental assessment	Stanford/Binet	Bayley	Denver/Griffiths
Defaulters chased?	No	Yes	Yes
Transport provided	No	Yes	By volunteers

TABLE III.11.3

Laboratory tests available

	Padua	Berlin	Edinburgh
HIV Ab	Yes	Yes	Yes
HIV Ag	Pos/neg	Pos/neg	Quantitative
p24 Ab	No	No	Quantitative
Western blot	Routine	Not routine	Not routine
PCR	Experimental	Experimental	Experimental
HIV isolation	Yes	? reliable	Yes
IVAP	Yes	No	No

TABLE III.11.4

Details of HIV seropositive children followed

	Padua	Berlin	Edinburgh
Total number	120	110	74
Prospective	80	84	56
Infected	31	10	11
Symptomatic	20	8	10
Transmission rate	19%	9%	5%

TABLE III.11.5

Clinical features of the HIV infected children

	Padua	Berlin	Edinburgh
Median onset of symptoms/mths (range)	6 (1-60)	5(3-42)	12(1-36)
No. died	4	0	5
Age range survivors/mths	34-60	21-60	48-72
Median survival/mths	51	34	44
No. PCP	2	0	2
No. CMV	1	1	0
No. LIP	1	3	3
No. recurrent bacterial infections	3	2	2
Candida	1	0	6
Neoplasia	1	0	1
Encephalopathy	4	2	3

TABLE III.11.6

Laboratory features of infected children

	Padua	Berlin	Edinburgh
HIV Ab+	All	All	All
HIV isolation	25/25	10/10	10/11
HIV Ag +	23/31	3/10	9/11
IVAP	8/9	-	-
Raised IgG	17/20 symptomatic	8/8 symptomatic	11/11
Low IgG	0	0	0
CD4 count $<1.0x10^9/l$	10/31	3/10	10/11

SECTION IV

DISCUSSION

In this study, we aimed to identify all those children born to HIV seropositive women in the Edinburgh area, and by prospective follow-up, estimate the rate of perinatal transmission, and observe the natural history of HIV infection in this population. Information from antenatal and neonatal anonymous screening programmes (Tappin et al 1991) suggest that at the time of the study we were successful in identifying the population at risk. As we only included children followed prospectively from birth in the calculation of vertical transmission rate, we have prevented any bias towards subsequent identifcation of symptomatic (and therefore infected) children.

Diagnosis of HIV infection in infants who have maternally derived HIV antibody remains a major problem. We had hoped to study early indices of HIV infection from cord blood, and to this end, I was present at every delivery of an HIV seropositive woman between October 1987 and February 1990. During this time, only one child from the 30 deliveries of HIV seropositive women I attended proved to be infected, so that unfortunately, no conclusion could be reached.

There have been developments in diagnostic techniques by other workers. Although anti HIV IgM had proven disappointing (Parry & Mortimer 1986), IgA anti-HIV has been shown to have superior sensitivity to IgM, being present in 66% of infants subsequently proven to be infected, and in none who seroreverted (Weiblen et al 1990). Other

techniques, such as in vitro production of HIV specific antibody (Amadori et al 1988), and PCR (Ou et al 1988) look promising but these are still under evaluation and not routinely available. Because of the technical difficulty and the risks involved in obtaining and processing blood specimens from small babies, the possibility of diagnosis from alternative sources, such as saliva and urine have been explored, and saliva has been shown to be an adequate alternative using a sensitive test such as the *gag* ELISA (Burns et al 1991).

HIV isolation in culture and PCR are useful techniques, but remain research tools only available in a few centres. We did not identify any cases in addition to those already confirmed by conventional laboratory techniques, but we did not have the opportunity to evaluate a large series from samples taken in the early months of life, when other methods are less useful. There also remains an urgent need for improved methods to evaluate the seronegative index children who remain clinically well with normal immune function, as seven women have embarked on subsequent pregnancies, having observed that the index child was symptom free and antibody negative (presumed uninfected). Although PCR may prove a useful tool, it must be stressed that the demonstration on viral DNA sequences may not imply overt infection, and little is known about the immune response to HIV in children infected antenatally. As yet there have been no long term follow-up studies of children who have had blood analysed for PCR at birth or in the first few days of life to determine its reliability in predicting HIV infection status in the long term.

Candidates for other useful early markers include B_2

microglobulin and neopterin. B_2 microglobulin is a sub-unit of the human leucocyte class I antigen, and upon immune-system activation, is released by both T and B lymphocytes into the circulation (Chan et al 1990). Neopterin is produced and released by macrophages following stimulation by gamma interferon, and is closely correlated with activation of cell mediated immunity (Fuchs et al 1988). Elevated levels of both substances have been shown to be associated with advanced HIV disease (Lacey et al 1987, Abita et al 1985), and more recently have been shown in adults to occur early in the course of HIV infection (Sonnerborg et al 1989). In children, the normal range of these 2 markers is wide (Milman & Gutteberg 1987, Shintaku et al 1982), and may be raised by intercurrent viral infections (Cooper et al 1984). However, persistently high concentrations in CDC stage P0 patients has been suggested to be indicative of HIV infection (Chan et al 1990). Whether these markers have any additional value over other indices in the first year of life, however, remains to be seen.

This study was commenced at a time when the estimated rate of perinatal transmission of HIV was in the region of 50% (Scott et al 1984, Minkoff et al 1987). Later, prospective studies have quoted lower rates, but the rate of 5.4% that we have found was unexpected, and remains lower than any of the published series, although still within the confidence intervals of the European Collaborative Study (ECS 1991). From the point of view of the study, it is disappointing that although we have followed the largest group of infants born to HIV seropositive women in the UK, the resulting numbers of HIV infected children has been small. This has limited our ability to study prospectively the natural history of paediatric HIV infection and to undertake controlled

therapeutic trials. In all the subsequent studies of the cohort comparing HIV infected with uninfected children, the numbers in each group have been unbalanced. The inclusion of other HIV infected children identified outwith the prospective study has enabled us to draw some conclusions regarding natural history and therapy, but studies in larger groups of children are required to verify and to explore these findings further.

In following infants born to HIV seropositive mothers, the physician's view of what is best for the child and the importance of research may be different from that of the parents. When the child is ill, the need for medical attention is usually recognised by all parties, but when the child is asymptomatic, it is not so obvious. As the study continues, there will be increasing numbers of older children, who are thought to be uninfected, but because of the lack of a perfect diagnostic test for HIV, absolute assurances cannot be made. At present it is felt that such children should continue to be seen. As they reach school age, they will ask questions themselves about the reason for attending hospital, which their parents will find difficult to answer.

It is likely that most children infected with HIV from their mothers acquire the infection in utero. HIV has been isolated from amniotic fluid (Mundy et al 1987), placenta, (Hill et al 1987), and foetal tissue at 8-20 weeks gestation (Jovaisas et al 1985, Sprecher et al 1986, Lyman et al 1990, Lewis et al 1990), and CD4+ cells susceptible to infection have been identified in placental tissue (Maury et al 1989). The possibility of perinatal transmission from contact with infected cervical secretions (Pomerantz et al 1988), or postnatally from breast milk (Ziegler et al 1985, Lepage et al 1987, ECS 1992) also exists. The low

rate of Caesarean section, and the very small numbers of women breast feeding meant that we were unable to explore the additional risk to the child of exposure to either of these possible sources of HIV infection.

The low number of infected children in our series has led us to look at factors which might influence the risk of transmission, both within a population of HIV seropositive women, but also between cohorts. We were able to show that women who produced children shortly after seroconversion, and also those with more advanced disease, defined by symptomatic disease at the time of pregnancy, or by low CD4 counts, were more likely to transmit HIV to their offspring. These observations in addition to studies showing that high titres of antibodies to portions of gp120 may be protective (Goedert et al 1989, Rossi et al 1989) suggest that the development of, and later deterioration in the maternal immune response to HIV as disease progresses is likely to result in transmission. Much is yet to be discovered regarding the host immune response to HIV which may be relevant in the understanding of maternal factors influencing vertical transmission.

Foetal factors may also determine the susceptibility of the foetus to infection, as illustrated by case 3, whose dizygotic twin proved uninfected. Discordance for HIV infection between twins is well recognised (Menez Bautista et al 1986, Thomas et al 1990), and is not unique to HIV, being also described in congenital rubella (Forrester et al 1966). It has been suggested (Thomas et al 1990) that the smaller twin is more likely to be more seriously affected, but in our case the infected twin was the larger. A recent multicentre study (Goedert et al 1991) has suggested that the first twin

is more likely to be affected, especially if delivered vaginally, as was case 3. This finding has raised the question whether the discordance is not due to foetal differences in susceptibility, but the different perinatal events such as length of time in contact with the birth canal and maternal blood and secretions.

The median survival in our cohort was 67 months, which is longer than that reported by Scott et al (1989). However, our cohort is largely followed from birth, whereas many of Scott's patients were referred because of symptoms. A lower proportion of children presenting with early onset opportunistic infections or severe encephalopathy, who have a worse prognosis than those without these features (Blanche et al 1990). Although the number of opportunistic infections was low, many of the manifestations of HIV infection have been illustrated by our group, and much practical experience gained. Also, collaboration with other centres in Europe has led to useful epidemiological data being accumulated (ECS 1991).

We have described the prognostic value of serial measurement of CD4 counts, HIV Ag and p24 Ab levels. The absence of antibody with neutralising activity against HIV has been shown to be associated with disease progression (Weber et al 1987,Sei et al 1988), and children with measurable neutralising antibody were those who were clinically stable, most having LIP (Robert-Guroff et al 1987). The frequency of antibodies mediating cellular cytotoxicity is also lower in those children with AIDS than infected children without AIDS (Ljunggren et al 1990). More recently, clinical outcome has been shown to be related to titres of antibodies which inhibit syncytium formation, children with LIP having significantly

higher titres than those with opportunistic infection (Brenner et al 1991). Such studies use experimental techniques, however, and their application in routine clinical practice remains to be evaluated.

We have also explored the hypothesis that genetic factors may influence HIV disease by studying HLA types among the children, and concluded that differences in the prevalence of HLA types between populations may be related to the observed differences in transmission rates. However, the HLA type of an individual child born to an HIV seropositive woman is not likely to be helpful in predicting its risk of being infected.

Although much knowledge has been accumulated over the period of this study in the treatment of HIV children, therapeutic options remain limited, and those so far available appear to delay, rather than arrest the progress of disease. The benefit we have reported of IVIgG infusions in HIV infected children with recurrent bacterial infection has very recently been confirmed in a large multi-centre placebo controlled trial (NICH IVIgG study Group 1991). Although no difference could be demonstrated in survival, for children with CD4 lymphocyte counts $>200x10^6/l$, there was a significant reduction in serious bacterial infection, and in the number of acute hospital admissions. Further studies are needed, however, to define more accurately whether there is a sub-group of children most likely to benefit from therapy.

Zidovudine administration is now well established in clinical practice for children with symptoms of HIV infection. Remaining

questions include when to start treatment and the optimum dose to give. In adults, it has been shown that a lower dose of 500mg daily is equally effective and less toxic than that of 1500mg daily originally recommended, but the minimum effective dose is still to be established (Volberding et al 1990).

Trials are under way to determine at what time zidovudine should be commenced. One American study was terminated by the ES National Institute of Allergy and Infectious Disease after preliminary data suggested that initiation of therapy in those with CD4 counts less than 500/mm^3 delayed progression to symptomatic disease (Volberding et al 1990). However, the Anglo-French 'Concorde' trial, which is a multi-centre trial comparing AZT and placebo administration in asymptomatic HIV seropositive adults, continues (Editorial, Lancet 1989). One concern over early commencement of treatment is the development of zidovudine resistance, which is related to the duration of therapy (Larder et al 1989). Such mutant strains can be detected after 6 months of therapy, and mutations may occur more rapidly in those who progress to AIDS (Boucher et al 1990). Early use may encourage the selection of zidovudine resistant strain in the future, with potential loss of benefit of the drug once clinical illness occurs (Friedland 1990), and the use of combinations of antiretroviral drugs may be required to control selective zidovudine resistant variants (Rooke et al 1989).

Another possible use of anti-retroviral therapy is to reduce the risk of vertical transmission of HIV. Administration of zidovudine to transgenic mice has been shown to prevent viraemia in their offspring (Sharpe et al 1988). Transfer across the placenta in human has been

demonstrated (Liebes et al 1990) with good levels produced in foetal tissue, and there are reports of zidovudine being tolerated well during pregnancy, with no adverse effects on the foetus (Chavanet et al 1989). However, the amount of protection from transmission afforded by such intervention is unknown, and further experience may yet indicate an unacceptable level of foetal toxicity.

It is clear there is a requirement for other modes of therapy to be developed. Preliminary trials using 2',3'- dideoxycytidine(ddC) have shown some in vivo effect, with a different toxicity profile to zidovudine (Yarchoan et al 1988, Merigan et al 1988), but the purine 2',3'-dideoxyinosine (ddI) has proved the most promising of the other 2',3' dideoxynucleosides. Unlike zidovudine, haematological toxicity is not a problem, but peripheral neuropathy, pancreatitis, and hepatitis may result from dose above 9.6mg/kg/day (Yarchoan et al 1990). One further advantage is that it can be given once a day (Cooley et al 1990), and ddI has been shown to have in vitro activity against zidovudine resistant strains of HIV (Larder et al 1989). On a twice daily regime, decreases in serum p24 Ag, and rises in CD4 counts at up to 20 weeks have been demonstrated (Lambert et al 1990). DDI has also been shown to cross the placental barrier but the effect on the foetus is unknown (Pons et al 1991).

Interruption of other steps in the cycle of HIV replication may also be possible. Soluble CD4, which blocks the initial binding of gp120 *env* protein of HIV to CD4 has been studied intensively (Fisher et al 1988, Hussey et al 1988, Deen et al 1988, Capon et al 1989, Perno et al 1990). Clinical trials have shown no significant clinical or immunological toxicities, despite the concern that excess quantities of CD4 could disrupt immune function

(Kahn et al 1990, Schooley et al 1990, Editorial, Lancet 1990). However, its half life is short at 1 hour (Kahn et al 1990), and macrophages may still become infected through CD4 independent mechanisms (Broder 1990); brain and muscle cells may be unprotected (Clapham et al 1989).

Other agents shown to have some anti HIV activity have included fusidic acid (Lloyd et al 1988, Faber et al 1987) and dithiocarb (Lang et al 1988, Reisinger et al 1990). It is likely that treatment of HIV in the future will depend on the development of more effective combinations of drugs, as has been the case with haematological malignancies, rather than the discovery of a 'miracle cure'. New compounds to use alone or in combination are therefore required if survival is to be further prolonged.

Efforts have already been made to control the further spread of HIV by screening blood products, provision of clean needles, and education programmes promoting safe sexual practices (Chin 1990). However, a programme of prevention involving major changes in life-style is not likely to enjoy universal application. In the future, one hope for the control and eradication of this disease lies in the prospects for the development of a safe and effective vaccine, not only for prophylaxis against primary HIV infection, but also for immunotherapeutic use to delay the progression of clinical disease (Schild & Minor 1990). Vaccine development, however, has proven problematical, not only because the virus infects the very cells required to mount an immune response (Laurence & Schild 1989), but also because of the requirement to find suitable animal models (Newmark 1989). Because of the long latency between HIV infection and clinical disease, the use of

live attenuated viruses similar to those used in other viral infections is precluded by virtue of the fact that it will be impossible to assess their safety before use (Schild & Minor 1990). Attention is therefore being focused on development of subunit preparations based on recombinant DNA technology, which also provided an effective hepatitis B vaccine. Suitable viral subunits are likely to be the *env* and *gag* proteins. Antibodies against *env* protein are able to neutralise viral infectivity (Bolognesi 1989), and high levels of antibody to *gag* is associated with a prolonged symptom free period (Lange et al 1989), but results of preliminary tests on volunteers are awaited, and suitable adjuvants still need to be found (Koff & Fauci 1989).

Meanwhile, AIDS remains a devastating terminal disease, affecting children who may already be socially disadvantaged. Advances in the management of these children, therefore, not only involves research into diagnosis and treatment, but also into methods of support for them and their families, many of whom have other affected members. This will not only involve material, psychological, and spiritual support for the families themselves but also addressing the issues of confidentiality, ostracism, peer group education, and education of the general public. While we strive to improve the quantity of life for these children, at least equal attention needs to be paid to its quality.

SECTION V

CONCLUSIONS

We have described the methods used and results obtained from this study designed to answer questions a-g posed in the introduction. Our conclusions, relating to those questions can be summarised as follows:

a) The risk of virus transmission from an HIV positive mother to her offspring among a population of Caucasian women, the majority of whom have a history of intravenous drug use and are asymptomatic at the time of pregnancy is 5.4% (95%CI 1.12-14.87%).

b) There are no clearly identifiable genetic factors influencing vertical transmission of HIV. HLA antigens may be associated with susceptibility to transmission, and in particular, a high A3, B8, DR2/A1, B8, DR3 ratio may contribute to a low transmission rate, but this hypothesis needs to be explored further.

Important maternal factors influencing transmission are the timing of pregnancy in relation to seroconversion, and the stage of maternal disease. Pregnancies in the first year of seroconversion are more likely to result in HIV infected offspring. Progression of clinical disease in the mother and a low CD4 count during pregnancy may also increase the risk to the foetus. There may be other factors also influencing the risk of perinatal transmission, such as maternal HIV antigenaemia, which failed to reach significance in our study, and

require follow-up of larger groups of women and children to identify. Similarly, perinatal factors, such as mode of delivery or method of feeding could not be explored because so few women underwent Caesarean section or breast fed.

c) In our experience, early clinical indications of HIV infection include recurrent respiratory infections, recurrent diarrhoea, eczematous skin rashes, generalised lymphadenopathy, and hepatosplenomegaly. Although candidiasis in infancy was not significantly more frequent in HIV infected children, persistent severe candidal infection has been demonstrated to herald the development of AIDS. Useful laboratory indices in the first 18 months of life include IgG, which may be raised by 6 months of life, and HIV Ag, which was positive in the first 18 months in all five children on whom data is available. HIV isolation in culture and PCR may provide the diagnosis, but negative results, however, are unhelpful. HIV antibody is the most useful test in older children.

d) In children proving to be HIV infected, median time for development of HIV related symptoms was 12 months; 55% had symptoms by a year of age (95% CI 23.38-83.25%), and 82% by 2 years (95% CI 48.22-97.72%). Median time of progression to stage P2 B-D was 45 months. One child remained asymptomatic at 42 months. Clinical features associated with progressive disease included PCP, recurrent bacterial infections, LIP, encephalopathy, oesophageal candida, Ewing's sarcoma, cardiomyopathy, and wasting syndrome. The development of encephalopathy was associated with a poor prognosis (100% mortality within 18 months), whereas LIP was

compatible with prolonged survival (maximum 5 years from inital diagnosis).

PCP was fatal in both cases. Rapid diagnosis from nasopharyngeal secretions without requiring invasive sampling was shown to be possible. Adherence to the CDC guidelines for PCP prophylaxis which have since become available would not have protected the child in our study from this infection, and it may be justifiable to treat all infants born to HIV seropositive women with cotrimoxazole until their HIV infection status is known.

HIV infected children suffer more symptomatic respiratory infections than those proving not to be HIV infected, and respiratory viruses can be isolated more frequently from their upper respiratory tracts. Respiratory infections are more likely to lead to hospital admissions in these children. Further studies are needed to explore the influence of intercurrent viral infection on the progression of HIV disease.

Both HIV infected children followed from birth who survived to 6 months were hypergammaglobulinaemic, as were all the other children at presentation. IgG peaked at a median of 28 (range 4-72) g/l at an age of 47 (range 4-63) months. Levels of IgM reflected IgG. Reverse CD4/CD8 ratio was not seen until a median age of 27 months (range 13-46 months), and CD4 lymphopoenia until a median of 27 months (range 4-46 months). Rate of decline of CD4 counts ranged from 0% to 99% per annum. In those followed from birth, decline in p24Ab level was associated with detection of HIV Ag in the serum, but as p24 Ab

levels rose, HIV Ag was not detected in the majority of children. Loss of p24 Ab and later detection of HIV Ag was seen later in the course of the disease, levels of >50pg/ml being predictive of poor outcome.

e) In young babies, severe candidiasis may herald the onset of AIDS, but the other early clinical features we observed were unhelpful prognostically. High levels of HIV Ag (>50pg/ml) in the absence of detectable p24 Ab were associated with progression of HIV disease, and mortality within 2 years. A rapidly declining CD4 count (>60% p.a.) similarly is a poor prognostic sign. Levels of IgG and IgM did not correlate with prognosis. The four children with raised IgA levels, however, showed rapid progression of disease with death within 2 years.

f) Intravenous immunoglobulin infusions given to children suffering from recurrent respiratory infection or recurrent diarrhoea leads to an improvement in weight gain, number of infections, and number of acute hospital admissions, and for this reason is a cost effective way of managing these children. Intravenous immunoglobulin therapy may also reduce the frequency of viral as well as bacterial infections. Zidovudine has been shown to be associated with improvement in CNS symptoms, and in those children given the drug on the basis of declining CD4 counts, stabilisiation of CD4 counts was achieved for 6 months, and a slower rate of decline observed thereafter. Numbers were too small for placebo controlled trials.

g) i) HIV infection in childhood is likely to carry a high mortality, although our follow-up is insufficiently long to determine whether the

infection is universally fatal. Mortality was 1/11, or 9% at 1 year (95% CI 0.23-41.28%), 3/11, or 27% at 5 years (95% CI 6.07-60.97%). 6/11 children died at median age of 55 months (95% CI 23.38-83.25%), range 4-82 months. The longest survivor is 76 months.

ii) Of those children presumed uninfected, none has shown symptoms or signs of HIV disease, and (with the exception of one fatality) all remain well. Maximum follow up is still only 74 months, however, and so these children need to be kept under review.

APPENDICES

APPENDIX I

Table I

Provisional case definition for AIDS surveillance of children (in use 1985 to 1987).

For the limited purposes of epidemiological surveillance, CDC defined a case of paediatric AIDS as the condition of a child with *both* of the following:

1) A reliably diagnosed disease at least moderately indicative of underlying cellular immunodeficiency

2) No known cause of underlying cellular immunodeficiency or any other reduced resistance reported to be associated with that disease

The diseases accepted as sufficiently indicative of underlying cellular immunodeficiency were the same as those used in defining AIDS in adults. In the absence of these opportunistic diseases, a histologically confirmed diagnosis of chronic LIP was considered indicative of AIDS unless test(s) for HIV showed negative results. Congenital infections (eg toxoplasmosis or herpes simplex virus infection in the first 6 months after birth) must have been excluded.

Specific conditions that must have been excluded in a child were as follows:

1) Primary immunodeficiency diseases: severe combined immunodeficiency, DiGeorge syndrome, Wiskott-Aldrich syndrome, ataxia telangiectasia, graft-versus-host disease, neutropoenia, neutrophil function abnormality, agammaglobulinaemia, or hypogammaglobulinaemia with raised IgM values

2) Secondary immunodeficiency associated with immunosuppressive therapy, lymphoreticular malignancy, or starvation

Modified from CDC:MMWR 1985; **34:** 517-21 by Falloon et al (1989)

Table 2

Summary of September 1987 revision of surveillance case definition for AIDS

Without laboratory evidence of HIV infection (test not done or inconclusive) a patient with AIDS:

A Does not have another cause of immunodeficiency, such as the following:

1) High dose or long term systemic corticosteroid therapy or other immunosuppressive-cytotoxic therapy <3 months before the onset of the indicator disease

2) Hodgkin disease, non-Hodgkin lymphoma (other than primary brain lymphoma), lymphocytic leukaemia, multiple myeloma, other cancer of lymphoreticular- histiocytic tissue, angioimmunoblastic lymphadenopathy < 3 months after diagnosis of the indicator disease

3) A genetic (congenital) immunodeficiency syndrome or an acquired immunodeficiency syndrome atypical of HIV infection (such as one with hypogammaglobulinaemia)

and

B Has had one of the following AIDS indicator diseases definitely diagnosed:

1) Candidiasis of the oesophagus, trachea, bronchi, or lungs
2) Extrapulmonary cryptococcosis
3) Cryptosporidiosis with diarrhoea persisting >1 month
4) Cytomegalovirus disease of an organ other than liver, spleen, lymph nodes in a patient >1 month of age
5) Herpes simplex virus infection causing a mucocutaneous ulcer persisting >1 months
   or bronchitis, pneumonitis, or oesophagitis in a patient >1 months of age
6) Primary lymphoma of the brain in a patient <60 years of age
7) Kaposi sarcoma in a patient <60 years of age
8) LIP and/or pulmonary lymphoid hyperplasia in a child <13 years of age
9) Mycobacterium avium complex or M. kansasii disease disseminated to site other than lungs, skin, or cervical or hilar lymph nodes
10) PCP
11) Progressive multifocal leukoencephalopathy
12) Toxoplasmosis of the brain in a patient >1 month of age

II With laboratory evidence of HIV infection, a patient with AIDS:
A Has had one of the already listed AIDS indicator diseases definitely diagnosed *or* one of the following AIDS indicator diseases definitely diagnosed:

1) Multiple or recurrent bacterial infections (at least 2 within 2 years) in a child <13 years of age, including septicaemia, pneumonia, meningitis, bone or joint infection, abscess of internal organ or body cavity (except otitis media or superficial skin or mucosal abscesses)
2) Coccidioidomycosis disseminated to a site other than lungs or cervical or hilar lymph nodes
3) HIV encephalopathy
4) Histoplasmosis disseminated to a site other than lungs or cervical or hilar lymph nodes
5) Isosporiasis with diarrhoea persisting >1 month
6) Kaposi sarcoma
7) Primary lymphoma of the brain
8) Other non-Hodgkin lymphoma of B cell or unknown immunologic phenotype (small, noncleaved Burkitt or non-Burkitt lymphoma or immunoblastic sarcoma)
9) Disseminated nontubercular mycobacterial disease involving a site other than lungs, skin, or cervical or hilar lymph nodes
10) Tuberculosis involving at least one site other than lungs
11) Recurrent nontyphoid *Salmonella* bacteraemia
12) HIV wasting syndrome

or

B One of the following AIDS indicator diseases diagnosed presumptively:

1) Oesophageal candidiasis
2) Cytomegalovirus retinitis with loss of vision
3) Kaposi sarcoma
4) LIP or pulmonary lymphoid hyperplasia in a child <13
5) Acid-fast infection (species not identified) disseminated to a site other than lungs, skin, or cervical or hilar lymph nodes
6) PCP
7) Toxoplasmosis of the brain in a patient >1 month

III With laboratory evidence against HIV infection (negative test), a patient with AIDS:

A Does not have another cause of underlying immunodeficiency

and

B Has had PCP definitely diagnosed

or

Has had definitive diagnosis of one of the AIDS indicator diseases listed in section I *plus* a T-helper lymphocyte count $<400/mm^3$

Table 3

World Health Organisation clinical case definition of AIDS in children

Major signs

Weight loss or failure to thrive
Chronic diarrhoea (>1 month)
Prolonged fever (>1 month)

Minor signs

Generalised lymphadenopathy*
Oropharyngeal candidiasis
Repeated common infections (otitis, pharyngitis, etc)
Persistent cough (>1 month)
Generalised dermatitis
Confirmed maternal HIV infection

Paediatric AIDS is suspected in an infant or child presenting with at least 2 major signs associated with at least 2 minor signs in the absence of known causes of immunosuppression.

* Generalised lymphadenopathy = lymph nodes measuring at least 0.5cm and present in 2 or more non- contiguous sites

Table 4

CDC case definition of HIV infection in children less than 13 years of age

1) Children <15 months of age with perinatal infection have *one* of the following:

a) HIV in blood or tissues confirmed by culture or other laboratory detection method
b) Symptoms meeting CDC case definition for AIDS
c) Antibody to HIV (repeatedly reactive screening test plus positive confirmatory test result) *and* evidence of both cellular and humoral immunodeficiency (increased immunoglobulin levels, decreased absolute helper T cell count, absolute lymphopoenia, reduced helper-suppressor T cell ratio *and* symptoms (class P2)

2) Older perinatally infected children or children who acquired infection through another mode of transmission have *one* of the following:

a) HIV in blood or tissues confirmed by culture or other laboratory detection method
b) Antibody to HIV (repeatedly reactive screening test plus positive confirmatory test result)
c) Symptoms meeting CDC case definition for AIDS

Modified from CDC MMWR 1987; **36:** 225-36

Table 5
Classification system for HIV infected children less than 13 years of age

P0 Indeterminate infection in perinatally exposed children younger than 15 months of age who have antibody to HIV.

P1 Asymptomatic infection

A Normal immune function. Normal immunoglobulins, FBC, lymphocyte subsets

B Abnormal immune function.

One or more (unexplained): hypergammaglobulinaemia, T helper cell lymphopoenia, decreased T helper/T suppressor ratio, absolute lymphopoenia C Immune function not tested

P2 Symptomatic infection (causes other than HIV excluded)

A Nonspecific findings.

More than 2 months persistence of 2 or more (unexplained): fever, failure to thrive, or >10% weight loss, hepatomegaly, splenomegaly, generalised lymphadenopathy (as above), parotitis, diarrhoea with 3 or more loose stools daily persistently or recurrently (2 or more episodes with dehydration within 2 months)

B Progressive neurological disease.

One or more loss of developmental milestones or intellectual ability; impaired brain growth (acquired microcephaly and/or brain atrophy on scan); progressive symmetrical motor deficits with 2 or more of paresis, abnormal tone, pathologic reflexes, ataxia, or gait disturbance.

C Lymphoid interstitial pneumonitis Histologically confirmed with diffuse interstitial and peribronchiolar infiltration of lymphocytes and plasma cells without identifiable pathogen *or* chronic pneumonitis with bilateral reticulonodular infiltrates with or without hilar adenopathy on chest radiograph for at least 2 months, unresponsive to antimicrobial therapy and without other identifiable pathogen

D Secondary infectious disease

D1 Those listed in CDC definition of AIDS PCP; chronic cryptosporidiosis; disseminated toxoplasmosis (onset after 1 month of age); extraintestinal strongyloidosis; chronic isosporiosis; candidiasis (oesophageal, bronchial, or pulmonary); extrapulmonary cryptococcosis; disseminated histoplasmosis; noncutaneous, extrapulmonary or disseminated mycobacterial infection (species other than leprae); CMV infection (onset after 1 month of age); extrapulmonary or disseminated coccidioidomycosis; nocardiosis; progressive multifocal leukoencephalopathy; chronic mucocutaneous or disseminated HSV infection (onset after 1 month of age)

D2 Recurrent serious bacterial infections Two or more within 2 years: sepsis, meningitis, pneumonia, abscess of internal organ, bone or joint infection

D3 Other infections Oral candidiasis (persisting 2 months), 2 or more episodes of herpes stomatitis within 1 year, multidermatomal or disseminated herpes zoster

E Secondary cancers

E1 Those listed in CDC definition of AIDS Kaposi sarcoma, B cell nonHodgkin lymphoma, primary lymphoma of brain

E2 Others

F Other diseases possibly caused by HIV infection Hepatitis, cardiomyopathy, nephropathy, haematological disorder (anaemia, thrombocytopoenia), dermatological disease

Modified from CDC (1987). *MMWR* **36:** 225-36

Table 6

Recommended indicator levels of CD4+ lymphocytes for the initiation of prophylaxis for PCP are:

$<1.500 \times 10^6$/l for children 1-11 months
$<750 \times 10^6$/l for children 12-23 months
$<500 \times 10^6$/l for children 24 months to 5 years
$<200 \times 10^6$/l for chidlren 6 years and older

Table 7

Drug regimens for PCP prophylaxis

1. Recommended regimen (children >1 month of age
Trimethoprim/sulfamethoxazole (TMP-SMX) 150mg TMP/m^2/day with 750mg SMX/m^2/day given orally in divided doses twice a day 3 times per week on consecutive days.

Acceptable alternative TMP-SMX dosage schedules:
A 150mg TMP/m^2/day with 750mg SMX/m^2/day given orally as a single daily dose 3 times per week on consecutive days.
B 150mg TMP/m^2/day with 750mg SMX/m^2/day orally divided bd and given 7 days /week
C 150mg TMP/m^2/day with 750mg SMX/m^2/day given orally divided bd and given 3 times per week on alternate days.

II Alternative regimens if TMP-SMX not tolerated

Aerosolised pentamidine (>5 years of age) 300mg given via Respirgard II inhaler monthly.

Dapsone (>1 month of age) 1mg/kg (not to exceed 100mg) given orally once daily.

If neither is tolerated, some clinicians use intravenous pentamidine (4mg/kg) given every 2 or 4 weeks.

APPENDIX II

CASE REPORTS

Case 1

Born at 42 weeks gestation by spontaneous vaginal delivery after an uneventful pregnancy, she weighed 3.25kg at birth. Her mother had used intravenous drugs and was known to be HIV seropositive. At six months she was admitted for a week with an upper respiratory tract infection, improving after treatment with amoxycillin. Parainfluenza virus type 3 was isolated from nasal secretions. At that time she had bilateral axillary lymphadenopathy and a 3cm palpable liver. A month later she was admitted for 2 weeks with measles and an inflamed right eardrum. She was treated with hyperimmune measles immunoglobulin and erythromycin and improved.

At 9 months she was failing to thrive and was admitted with a week's history of vomiting and watery diarrhoea. She was miserable, irritable and had a purulent nasal discharge. Her weight was 6.5kg (below 3rd centile) and she had an inflamed right eardrum. There was generalised lymphadenopathy and hepatomegaly with no splenomegaly. She failed to improve and after a week developed a Haemophilus influenzae pneumonia. No pathogenic organisms were isolated from the stools. She was treated with erythromycin and 3 weekly IVIgG infusions and improved. There was a particular improvement in her mood on the morning following her first infusion; she became alert with spontaneous laughter for the first time. She was discharged after 5 weeks, and thereafter she began to gain weight and remained free of infections sufficiently severe to merit hospital admission.

She continued on her IVIgG infusions but although clinically she remained stable, her CD4 count began to decline. By 28 months of age the count was 600×10^6/l. She was commenced on oral zidovudine for this reason, and tolerated the drug well. No side effects were noted. At 39 months, she developed widespread molluscum contagiosum over her arms, face and trunk. No specific treatment was given, and the lesions gradually resolved over the next 6 months. Since than she has remained well, and continues to thrive. Her neurological development remains within normal limits, and she attends normal school.

Case 2

This infant was born at 38 weeks gestation by forceps delivery after an uneventful pregnancy, weighing 3.33kg. Her father, but not her mother had a history of IV drug use. Neonatal jaundice responded to phototherapy and she was discharged at 6 days, only to be readmitted for social reasons, her mother having developed hepatitis B.

At two months, she was admitted to hospital with rotavirus gastroenteritis, and at 10 months she was admitted for 12 days with cough, bilateral otitis media and a glandular fever-like illness, with lymphadenopathy, hepatosplenomegaly, and 12% atypical mononuclear cells in the peripheral blood. EBV serology was negative. She improved, but was again admitted for 3 days a month later because of a troublesome cough. At the age of 12 months she was admitted with right lower lobe pneumonia, which resolved after antibiotic treatment, although no organisms were isolated. There were two further admissions over the next 5 months because of a troublesome cough attributed to previous pertussis infection, vaccination having been declined.

HIV infection was confirmed at 17 months, when both parents were also diagnosed. At 23 months she continued to have recurrent upper respiratory infections

and impetigo, not requiring hospital admission. Her height and weight were on the 50th centile. A purulent nasal discharge and peri-oral impetigo were present, with generalised lymphadenopathy, and hepatosplenomegaly.

She was commenced on IVIgG at 24 months, and in the following year had two episodes of diarrhoea and occasionally a runny nose. She was admitted to hospital once with vomiting, diarrhoea, and pyrexia, all of which settled spontaneously with no pathogens isolated. There was an improvement in mood similar to that in case 1. Over the next year she had three brief admissions for diarrhoea, and for social reasons. Her mother developed PCP when she was 3 years of age.

At 42 months, she developed widespread molluscum contagiosum infection, associated with a sudden fall in her CD4 count to $50x10^6/l$. She was commenced on oral zidovudine and although for the first 2 weeks she complained of headaches and sore legs, thereafter she tolerated it well. At 48 months she was admitted with impetigo over the scalp treated effectively with topical preparations and oral antibiotics. Her mother died of cerebral toxoplasmosis when she was 54 months, and she was looked after by paternal grandparents.

At 69 months she was admitted complaining of breathlessness, dizziness, and reduced exercise tolerance. She was tachypnoeic, tachycardic, and was found to have cardiomegaly. Cardiomyopathy was diagnosed on the basis of echocardiography: endomyocardial biopsy was unhelpful in defining the aetiological agent. She was treated initially with digoxin and diuretics, but 3 months later, captopril was introduced. Thereafter she had increasingly poor exercise tolerance, and having previously been well nourished, she began to fail to thrive. She was finally admitted at the age of 82 months in terminal cardiac failure, and died 5 days later. Permission for post mortem was not given.

Case 3

The first of dizygotic twins born at 37 weeks, weighing 2.18kg; her mother was known to have shared needles, and worked in the sex industry. Her neonatal course was complicated by jaundice requiring phototherapy, and by a chlamydial eye infection. At three months of age she was admitted with a persistent cough, and purulent nasal discharge. She also had otitis media, which was treated with antibiotics.

At 14 months she presented pyrexial and lethargic, with a history of chronic otitis media. She had a generalised maculopapular rash, compatible with measles, although no organism was isolated, and had generalised lymphadenopathy and hepatosplenomegaly. She was commenced on IVIgG. She had one further admission at 18 months of age with a viral illness, but remained otherwise well. Her weight gain parallelled that of her twin sister.

At 27 months, she developed widespread bruising and her platelet count was low, at $20x10^9/l$. At this time she also had persistent cough and fever, and a chest Xray showed appearances typical of LIP. Because of recurrent bruising and platelet counts persistently below $20x10^9/l$, she was commenced at 50 months of age on high dose IVIgG (1g/kg), and later on anti D. She remains otherwise well, and has now started at her local primary school.

Case 4

The mother of this female infant developed PCP at 35 weeks gestation and the baby was delivered at term weighing 3.05kg. There were no neonatal problems. Cord blood was positive for HIV Ab on ELISA, negative for HIV antigen, HIV p24 Ab, and viral isolation. CD4 count was 2.34×10^9/l and her initial progress was uncomplicated. By one month she developed persistent candidal infection of the oral cavity and napkin area, and by 2 months she had become HIV Ag positive. Her CD4 count at this time was 1.94×10^9/l.

At four and a half months she presented with a two week history of mild upper respiratory symptoms, with two days of pyrexia, an unproductive cough, and increasing tachypnoea, sufficiently severe to impair feeding. Examination revealed a pale, mildly cyanosed and lethargic, but responsive infant. She had a tachycardia of 160/min and respiratory rate of 80/min, with bilateral intercostal and subcostal indrawing and scattered crepitations on auscultation. Lymphadenopathy and hepatosplenomegaly were noted, along with candidal dermatitis of the napkin area.

The chest X-Ray showed extensive bilateral pneumonitis. She was hypoxic (oxygen saturation 60%), with a pCO2 of 5.37kPa and hydrogen ion of 39.7mmol/l. On admission the neutrophil count was 6200/cu mm, CD4 1.79×10^9/l and CD4/CD8 ratio, 1.4. Serum IgG was normal (4g/l), and IgA and IgM elevated (0.9g/l and 2.1g/l). HIV Ag was positive (140pg/ml). Secretions obtained by nasopharyngeal aspiration were sent for microbiological investigation.

Pending results the infant received oxygen supplementation and high dose cotrimoxazole and acyclovir intravenously. The identification of pneumocystis carinii was confirmed the following day (see chapter 6). The infant's condition initially stabilised, but deteriorated 24 hours later. Despite giving hydrocortisone and intravenous immunoglobulin, she failed to maintain oxygen saturation of >80% even in 100% FiO2, and developed CO2 retention (pCO2 9.75kPa) and respiratory acidosis ($[H^+]$ 73.7mmol/l). Definite knowledge of the diagnosis, and its implications for the child's long term prognosis meant that the parents could be counselled appropriately. Their wish for her not to receive mechanical ventilation was respected, and she died on the fourth day of her admission. Post mortem confirmed the widespread alveolar exudate within the lungs in which round organisms were detected by silver staining. No other organisms were detected. Her mother died 18 months later from cerebral toxoplasma having suffered multiple cerebrovascular accidents and a further episode of PCP.

Case 5

This female infant was born at 37 weeks gestation weighing 2.8kg. Mother's partner had a history of intravenous drug use, but the mother herself had never shared needles. Neonatal course was uneventful and she was discharged at 2 days. In the first year of life she had recurrent wheeze and eczema, but was otherwise well.

The diagnosis of HIV infection was made when she was 21 months, mother having been identified on antenatal screening during a subsequent pregnancy. At this time, she had generalised lymphadenopathy and hepatosplenomegaly, and had had some diarrhoea. These all resolved, however, and although persistently HIV antigenaemic, she remains p24 Ab positive and asymptomatic. She attends her local nursery school.

Case 6 reported by Forsyth et al (1987)

This 29 weeks' gestation baby weighed 930g at birth. Both parents had a history of intravenous drug use. He required ventilation for a few days, and was given phenobarbitone prophylactically but no withdrawal symptoms developed. He developed intestinal obstruction at 10 weeks of age due to a right inguinal hernia, which was repaired. He was discharged to the care of foster parents thereafter.

He remained well until at 8 months he was admitted with a right upper lobe pneumonia, successfully treated with ampicillin and flucloxacillin. Three months later he began to develop petechiae and was noted to bruise easily. At 13 months he was admitted for investigation , and was found to have a platelet count of 16×10^9/l, with otherwise normal coagulation. It was at this time that the diagnosis of HIV was made. He received steroids, and high dose intravenous immunoglobulin, after which his platelet count fluctuated between 20 and 50 $\times 10^9$/l. He continued to bruise easily, but never developed major haemorrhage.

He subsequently developed generalised lymphadenopathy. He had recurrent upper respiratory tract infections, and intermittent episodes of diarrhoea. He required emulsifying cream for eczema, and intermittent Slophylline for wheeze. At 2 years of age he developed oral thrush which responded to Nystatin. At 33 months, he began to complain of pain in his right leg, with stiffness and a limp. His left eye was also drooping. There was little to find on formal neurological examination, however, and CT scan of the brain was normal. These symptoms resolved spontaneously.

At 37 months he commenced regular IVIgG infusions, and subsequently remained very well, with few problems, other than mild upper respiratory tact symptoms and no requirements for admission to hospital. Chest X ray appearances were however, consistent with lymphocytic interstitial pneumonitis. He had persistent HIV antigenaemia, and because of this, infusions of plasma rich in HIV core antibody were commenced, which he received from 51-57 months. He was started on zidovudine at 58 months, when he was still free of symptoms, but growing poorly. He developed recurrent coughs and pyrexiae, had intermittent parotid swelling, and had a parainfluenza B respiratory infection at 49 months.

By 67 months, he was anorexic, with nausea and vomiting. His weight was static, and his mother noted that he was falling a lot, although again there was little to find on neurological examination. Because of a falling CD4 count he was commenced on oral cotrimoxazole, which he failed to tolerate, so he was given regular nebulised pentamidine.

At 74 months, a painless mass was noted over the head of the left fibula. Radiography showed destruction of the metaphyseal region of the bone, with expansion and erosion of the cortex, and associated soft tissue swelling. This was diagnosed as a Ewing's sarcoma. No other bony abnormalities were found on technetium bone scan or limited skeletal survey, and no chest metastases were evident.

He was commenced on a modified regime of chemotherapy, consisting of vincristine, actinomycin, and etoposide, but only received two doses of etoposide, due to a severe rash and constitutional upset. The second pulse of chemotherapy was commenced when he was still neutropoenic (0.72×10^9/l), so that only 2 of 3 doses of tenoposide and one dose of vincristine were given. Because of marrow suppression, zidovudine was discontinued. Two months later an excision was performed, but there was residual tumour in the capsule. He received a further course

of vincristine and actinomycin thereafter, but tolerated this so badly that further courses were not contemplated. During this time HIV Ag fell from 116pg/ml to 27pg/ml.

A month later he complained of lumbar pain, together with weakness down the right side. There was painful limitation of movement, but spinal radiographs and isotope bone scans were normal. A week later he developed headache, drowsiness, attacks of flushing, and a left lower motor neurone facial palsy. There was an increased cell count in the CSF (233 lymphocytes/ml), a protein of 2500mg/l, and glucose <0.5mmol/l (blood glucose 5.2 mmol/l) but no organisms, and no HIV Ag or Ab was detected. CT scan showed cerebral atrophy only, and MRI scan showed no focal lesions. Thereafter, he became increasingly encephalopathic, with fluctuating tetraparesis, cranial nerve palsies, thalamic pain, and short term memory loss. His condition deteriorated, and he became cachectic and comatose. He died at home at the age of 81 months. Post mortem permission was not given.

Case 7

This child was born at 38 weeks gestation to a known IV drug user, and weighed 2.82kg. Neonatal period was uneventful, but she developed a chlamydial eye infection at 2 weeks of age. She was discharged home initially, but was fostered from 6 weeks, and was later adopted. She was discovered to be hepatitis B Ag positive after her foster mother contracted hepatitis, and at this time HIV infection was also diagnosed. At a year of age she was noted to have generalised lymphadenopathy, but she remained otherwise well until three years, when she presented with a 4 month history of recurrent diarrhoea, from which Campylobacter had been isolated on one occasion. She also had candidal infection of the perineum, and complained of lethargy, sleeping up to 5 hours during the day and 12-14 hours at night.

At 39 months she was commenced on IVIgG, had no further episodes of diarrhoea, and her sleep requirement diminished dramatically. Thereafter she remained well, with only one episode of respiratory infection, with cough and left lower lobe changes on X ray, but this responded to oral antibiotics. IVIgG was discontinued when she was 5 years of age and she remained well thereafter.

Case 8

The second of four children, she was born by spontaneous vaginal delivery at 43 weeks gestation weighing 3.15kg. Her mother received blood after the birth of her first child 2 years previously but the donors were traced and are HIV seronegative. There is no history of IV drug use. She was admitted at the age of 9 months, having been found near-drowned in a bath at home, from which she made a good recovery. At 28 months she was admitted for exploration of both groins as she was thought to have herniae. Inguinal lymphadenopathy only was found, which later resolved, the lymph node showing reactive hyperplasia. She had pertussis at 30 months and was admitted for 4 days with a UTI. At 37 months, she was admitted again with scalds to both her hands. During her admission she had an upper respiratory tract infection and Haemophilus was isolated. No chest X-ray was done at that time.

From 44 months she developed recurrent episodes of fever and cough, with both clinical and radiological evidence of bronchopneumonia. Mycoplasma pneumoniae and Haemophilus influenzae were isolated on different occasions from her sputum. She had also had persistent diarrhoea for the past five months. She was failing to thrive, her weight having fallen from the 50th centile at 18 months to less

than the third.

Initial investigations included a sweat test, which initially yielded an equivocal result with a sodium of 59mmol/l. Two repeat tests revealed sodium of 108mmol/l and chloride of 93mmol/l. Cystic fibrosis was assumed, and on this basis she was commenced on pancreatic supplements, and intensive physiotherapy and antibiotic treatment. There was no clinical improvement, and so the sweat test was repeated after 2 days of oral fludrocortisone, 2mg/m^2/day. Sweat sodium thereafter was 31mmol/l and chloride was 24mmol/l.

During her admission, at 47 months, she deteriorated, with pneumonia, weight loss and watery diarrhoea. Haemophilus was grown from her sputum along with Candida albicans, which was also in her mouth and pharynx. Oesophageal pain and barium studies were strongly suggestive of oesophageal candidiasis. Serological tests for PCP were negative. She responded to antibiotic and antifungal therapy. No organisms were isolated from stool specimens.

The diagnosis of HIV infection was made at this point, and she was commenced on IVIgG, and shortly after on AZT orally, 400mg/m^2/day. Over the next three months she improved, requiring 1 further admission for 3 days for a Haemophilus influenzae chest infection. AZT was stopped a week after this admission because of leucopoenia. Although AZT was recommenced, her compliance was poor, and she did not tolerate it well. She refused to take it by mouth because of the foul taste, and required an nasogastric tube for its administration. It is therefore unlikely that she received a therapeutic dose.

Over the next year her main problems were recurrent bacterial pneumonia, but she began to gain weight, and was well enough to start school with her peers. However, from 64 months she had unresolving widespread consolidation on chest X-ray. Despite physiotherapy and recurrent courses of oral and intravenous antibiotics, she became cachectic and died at 67 months. Post mortem was not performed.

Case 9

The younger sister of case 8 was born at 40 weeks gestation by spontaneous vaginal delivery. She was a face presentation. Birth weight was 2.75kg. Before the family was diagnosed as being HIV seropositive, at 12 months of age, she was admitted with a left lower lobe pneumonia, which was slow to resolve clinically and radiologically. Sputum was not obtained, but Strep.pneumoniae was isolated from blood cultures. She was initially treated with Ampicillin/flucloxacillin, but this was subsequently changed to benzylpenicillin.

She was 24 months when case 8 was diagnosed HIV seropositive. At this time she was thriving, with a few shotty lymph nodes, but otherwise well. A month later, however, she was admitted with pneumonia, initially responding in the absence of positive cultures to benzylpenicillin. However, later that months she was readmitted with similar symptoms. Gastric aspirates were cultured and grew Staph.aureus and Haemophilus influenzae. She again had patchy changes on the right, and consolidation at the left base on chest X-ray. She was treated this time with cotrimoxazole, and made a good clinical recovery. Residual radiological changes were suggestive of LIP.

At 26 months she was commenced on IVIgG. At this time she had extensive generalised lymphadenopathy and hepatosplenomegaly. She continued to thrive thereafter, and in the following year had only one episode of respiratory symptoms

requiring hospital admission. She had chicken pox, for which she was given acyclovir and hyperimmune Zoster immunoglobulin. She also had oral thrush. At 41 months she had herpes stomatitis, treated with acyclovir, requiring a 3 day admission, but its course was uncomplicated. She has had 2 further short admissions for exacerbation of respiratory symptoms, but radiologically, her LIP has not progressed further, and she has no chronic symptoms. When last seen at 76 months she was asymptomatic and thriving. She had small cervical lymph nodes only, her hepatosplenomegaly having regressed, and her chest was clear.

Case 10

The younger sister of cases 8 and 9 she was born by spontaneous vaginal delivery at 38 weeks gestation, weighing 3.04kg. She had chicken pox aged 11 months, at the same time as case 9, for which she received ZIG. The course of the illness was uncomplicated. At 12 months she was asymptomatic, but had generalised lymphadenopathy and hepatosplenomegaly. Over the next 9 months, although she showed a marked deterioration in CD4 count and was HIV Ag positive, she remained very well, continued to thrive, and her only additional findings were of oral thrush and some mild eczema. Her parents were unwilling for her to start any treatment on the basis of laboratory findings alone.

At 21 months she developed chronic purulent rhinitis. and shortly after, she began to have watery diarrhoea. IVIgG was commenced at 23 months, but she deteriorated rapidly. Enteropathogenic E Coli type 0128 was isolated from her stools. She developed candidal dermatitis in addition to oral candida, but no lower respiratory tract signs. She lost weight precipitously, becoming anorexic and cachectic. She was commenced on an IV infusion of zidovudine, with no demonstrable improvement. She showed marked developmental regression, losing speech completely. Gross motor function was impossible to assess because of her profound weakness. She died at 25 months, 2 weeks after case 8. Again, no post mortem was performed.

Case 11 (reported by Matthes et al (1988)

This child from Newcastle was born by spontaneous vaginal delivery at term weighing 3.15kg. He received the usual childhood immunisations, including measles, without problem and developed normally. He walked at 13 months, and by 21 months was starting to join words. At 21 months his mother was admitted with PCP, was found to be HIV seropositive, and died. HIV infection was then confirmed in this child and his father, but not his brother, aged 6. This mother received a blood transfusion after the birth of her first child, but the source of infection has not been confirmed.

He was well until aged 22 months, when he had a pertussis infection proven on culture requiring hospitalisation for 4 days. At that time he had generalised lymphadenopathy, and his weight had dropped below the 10th centile. Despite early treatment from day 7 of the illness with erythromycin, Bordetella pertussis could still be isolated at 28 days. A month later he had a unilateral submandibular lymphadenitis with Streptococcus pyogenes cultured from the fauces. This responded rapidly to drainage (culture negative) and intravenous benzylpenicillin.

At 26 months he had a severe pneumonia. The chest X-ray demonstrated consolidation in the right lower and middle lobes, with patchy shadowing in the upper lobe. Haemophilus influenzae was isolated from the sputum but he failed to respond to antibiotic treatment until given high dose cotrimoxazole, which produced clinical

and radiological resolution. Ten days after being admitted his speech rapidly regressed to expressive aphasia with only vowel sounds, but with no cognitive impairment. He also developed a considerable spastic diplegia with inability to walk. A CT scan showed cerebral atrophy. Sensory and motor nerve conduction studies gave normal results. His CSF showed no white cells and normal concentrations of glucose and protein. No bacteria, fungi, or viruses were cultured. IgG antibodies to HIV were selectively increased in the CSF. HIV Ag was not measured.

He was treated with IVIgG, initially weekly, then 3 weekly. A month later he was commenced on oral zidovudine 100mg/m^2 four times daily, equivalent to 16mg/kg/day. The intravenous preparation was given orally, and there were no early haematological or neurological toxic effects. Subsequently, his general well-being improved, he began to gain weight, and his neurological symptoms regressed. In particular, his spasticity reduced, so that he was able to run, and his reflexes returned to normal. He began to articulate several single words. A repeat CT after nine weeks treatment showed considerable improvement.

This improvement was sustained for about a year. He had a chronic cough, and his speech remained poor, but he was otherwise well. At 38 months he had a further admission with pneumonia, and at this time he was again unable to walk, or even pull himself to stand, and continued to have generalised lymphadenopathy and hepatomegaly. He also had a discharging ear. He was found to be pancytopoenic, and required platelet transfusion. No organism was isolated, but he received high dose cotrimoxazole and after 2 weeks, this was changed to dapsone. He was discharged 3 weeks later, having lost 3kg. A month later he developed persistent diarrhoea, and his general condition had deteriorated further. At 44 months he developed yet another pneumonia clinically consistent with PCP, to which he succumbed. Post mortem findings confirmed PCP.

Case 12

This baby's mother who had given birth 7 years earlier to an uninfected child, developed PCP during this pregnancy. There was also a history of heavy alcohol consumption throughout pregnancy. The baby was born at 38 weeks gestation weighing 2.17kg. From birth he had feeding problems, with irritability and vomiting and failed to thrive. At 3 months of age HIV was isolated from lymphocytes in culture, when HIV antibody was still positive. He is developmentally delayed and failing to thrive (weight at 9 months being 5.34kg), probably the result of the foetal alcohol syndrome. Although he has recurrent upper respiratory infections, he remains clinically well. HIV infection is manifested by persistent generalised lymphadenopathy only.

Case 13

This patient became infected with HIV during cardiac bypass surgery for transposition of the great arteries at the age of 4 months, before screening of blood for HIV antibody was available. He remained well until 14 months when he began to develop recurrent upper respiratory tract infections, and at 21 months of age developed ITP refractory to treatment with high dose IVIgG, and to prednisolone. At the age of 3 years 6 months, his platelet count had returned to normal, but he continued to have recurrent respiratory infections, failed to thrive, and had generalised lymphadenopathy and hepatosplenomegaly. Chest X ray showed appearances typical of LIP. The serum IgG, IgA and IgM levels were respectively 34.8, 2 and 2.8 g/l (normal ranges were 5-13, 0.47-2.63 and 0.36-1.92 g/l respectively). HIV infection was then diagnosed, based on retrospective screening of donor plasma and

subsequent HIV testing of the child. He was commenced on regular infusions of IVIgG when his serum IgG level was 50g/l.

Treatment with IVIgG was uneventful until his neurological reaction at the age of 4 years 10 months, described in detail in chapter 9, and apart from upper respiratory tract symptoms, and intermittent oral thrush, he remained well, and started at his local primary school.

At 5 years and 3 months he became more lethargic, falling asleep at school, and very reluctant to walk, with a mild ataxia but nothing more specific to find on general examination. He was found to be delayed on Griffiths developmental testing for gross motor function, but intellectually, he showed no deterioration. MRI scan showed evidence of cerebral atrophy and some minor changes in the white matter, but nothing focal. His CD4 count at this time was $0.3x10^9/l$. He was commenced on zidovudine, and showed improvement in gross motor function thereafter. At 5 and a half years he was diagnosed as having bilateral sensorineural deafness on routine screening at school, and was fitted with hearing aids. Intellectually, however, he kept well up with his peers at school, but fell further from the third centile for height and weight.

At six and a half years he developed persistent oral candidiasis. A month later he was found to have oral hairy leukoplakia. His height had been static for the past year at this point. At the age of 7, he became drowsy and lethargic. Neurological examination revealed blurred optic discs, pathologically brisk reflexes, and bilateral extensor plantar responses. CT scan revealed progressive cerebral atrophy, but there was no change on MRI. His zidovudine was increased to $720mg/m^2$/ day.

At 7 and a half years, he had a markedked decrease in exercise tolerance, with demonstrable hypoxia on exercise, although he remained 91% saturated in air at rest. This was attributed to LIP, and he was commenced on a course of oral prednisolone (2mg/kg/day initially), with some improvement. However, a month later he was admitted with a pyrexia. No focus could be found, but he still had oral thrush, and so fluconazole was commenced.

A month later, he was found to be in cardiac failure, with decreased right ventricular function on echocardiography. He was treated with frusemide, and his steroid dose was cut further. He remains on a maintenance dose of prednisolone.

APPENDIX III - Definitions of normal levels of laboratory indices

Immunoglobulin levels expressed in g/l

	IgG	IgA	IgM
Birth	5.0-13.0		
3 months	2.45-6.6	0.11-0.57	0.22-0.9
6 months	2.85-7.6	0.14-0.76	0.28-1.10
9 months	3.3-8.8	0.15-0.88	0.33-1.35
12 months	4.2-11.2	0.21-1.26	0.37-1.50
24 months	5.0-13.0	0.26-1.47	0.36-1.92

Normal ranges as defined by the Scottish National Blood Transfusion Service, based on analysis of samples from normal / asymptomatic infants and children (unpublished data)

Hypogammaglobulinaemia	below lower limit of normal for age
Hypergammaglobuliaemia	above upper limit of normal for age

Haematology

Neutropoenia	$<1.0 \times 10^9$/l neutrophils
Lymphopoenia	$<2.8 \times 10^9$/l lymphocytes
CD4 lymphopoenia	$<1.0 \times 10^9$/l CD4 lymphocytes in infants <18 months
Thrombocytopoenia	$<100 \times 10^9$/l platelets

APPENDIX IV

Proformata for recording of information are contained in the following five pages. The format of the ECS forms altered to a minor degree during the study, but the information recorded was the same throughout.

INTENSIVE PROSPECTIVE STUDY OF CHILDREN BORN TO HIV POSITIVE MOTHERS
MULTI-CENTRE EEC STUDY

MATERNAL INFORMATION AT DELIVERY

Centre 1-2
Mothers Study Number 3-5
Child Study Number 6

Mother's date of birth (day, month, year)
Country of birth 13-14

Marital Status

Single (1), Married (2), Divorced, Separated, Widowed (3), Cohabiting (4) 15

Ethnic Group

Asian (1), White (2), Black (3), Oriental (4), Other (5) 16
Age when leaving full-time education, years 17-18

Obstetric History

Number of previous livebirths 19-20
Number of previous stillbirths 21-22
Number of previous miscarriages 23-24
Number of terminations 25-26

Mothers Risk Group

History of Intravenous Drug Abuse (Y/N) 27
Trimester of last use: pre-conception (0), 1st (1), 2nd (2), 3rd (3), unknown (9) 28
Needle sharing? never (1) past (2) present (3) unknown (9) 29
Sexual partner of Bisexual (Y/N) 30
Sexual partner of Haemophiliac (Y/N) 31
Sexual partner of Intravenous Drug Abuser (Y/N) 32
Sexual partner of Other high risk group (Y/N) 33
(Specify) .....
Other 34

Mothers HIV History

Date of first HIV+ test (day, month, year)
SEROCONVERSION DATE

Current clinical status

Current HIV staging (CDC) 41-43
Specify symptoms 44-47
Date of onset 48-51
Specific HIV treatment in pregnancy? If so specify .....

MATERNAL CD4 COUNT
MATERNAL HIV Ag
MATERNAL p24 Ab
MATERNAL HIV CULTURE

7 1

ECS3
INTENSIVE PROSPECTIVE STUDY OF CHILDREN BORN TO HIV POSITIVE MOTHERS
MULTI-CENTRE EEC STUDY

PERINATAL INFORMATION

Centre 1-2

Mothers Study Number 3-5

Child Study Number 6

Child's date of birth (day, month, year)

Sex (M, F) 13

Gestational age (weeks) 14-15

Birthweight (gm) 16-19

OFC (cm) 20-22

Hospital where delivery took place 23-25

Obstetrician (initals) 26-28

Delivery

Caesarean Section: Elective (1), Emergency (2)
Vaginal: Spontaneous (3), Instrumental (4) 29

Scalp Electrodes (Y/N) 30
PLACENTA WT - NORMAL ?

Perinatal Problems (Y/N). Specify Details:

Hepatomegaly 31

Splenomegaly 32

Drug Withdrawal Symptoms 33

Thrombocytopenic Purpura 34

Infection: suspected (1) confirmed (2) 35

Transfusion 36

Congenital Abnormalities ..... * 37

Other ..... * 38

Disposition

with parents (1) fostered (2) adopted (3) 39

remained in hospital (4) other (5) .....

if remained in hospital, say why: ..... * 40

.....

Feeding: breast (1) bottle (2) breast and bottle (3) 41
was breast feeding tried and abandoned? Y/N

Died? Y/N 42

Date of death: (day/month/year)

Postmortem results, if available ..... * 49-52

.....

.....

Take sample required; Please record laboratory results on yellow form
Please store serum and cells at -70°C

7 2

INTENSIVE PROSPECTIVE STUDY OF CHILDREN BORN TO HIV POSITIVE MOTHERS
MULTI-CENTRE EEC STUDY

MEDICAL EXAMINATION

Please circle or complete as appropriate

~~Assessment at - 3w, 8w, 3m, 4.5m and 6m~~

Centre 1-2
Mothers Study Number 3-5
Child Study Number 6
Date of examination
Weight (kg) 13
Height (cm) 17
OFC (cm) 21-23

For c

24

Recurrent fever of unknown origin requiring medical attention ..... Y/N 25

Chronic or Recurrent diarrhoea requiring medical attention ..... Y/N 26

Specify organism 27

Bacterial infection ..... Y/N 28-30

If yes, specify: 31-33

Septicaemia, Meningitis, Urinary tract infection, Pneumonia, Other .....

Communicable Disease ..... Y/N 34-35

meales (1) mumps (2) rubella (3) varicella (4) zoster (5) Other (6) .....

Complications .....

Skin Infection requiring medical attention ..... Y/N 36-38

Staph (1) Strep (2) Herpes (3) Candida (4) Other (5) .....

Non-infectious skin eruption ..... Y/N 39

Petechiae/Purpura (1) Eczema (2) Kaposi Sarcoma (3) Other (4) 40

Palpable Lymph Nodes ..... Y/N 41-43

Axillary (1) Postoccipital (2) Cervical (3) Inguinal (4) Epitrochlear (5) Other (6) 44-46

Chronic parotid swelling ..... Y/N 47

Oral Candida persistent or recurrent despite therapy ..... Y/N 48

Upper repiratory tract infection ..... Y/N 49-50

chronic otitis media (1) sinusitis (2) chronic purulent rhinitis (3) Other (4) .....

Lower repiratory tract disease confirmed by X-ray ..... Y/N 51-53

Lymphocytic interstitial pneumonitis or pulmonary lymphoid hyperplasia (1)

pneumonia (2) bronchiolitis (3) Other (4) .....

specify organism, if known .....

Opportunistic infection ..... Y/N 54

PCP (1) CMV (2) Toxo (3) Candida (4) Mycobacterium (5) Other (6) .....

Hepatomegaly ..... Y/N 55

Splenomegaly ..... Y/N 56

7 1 57

PROSPECTIVE STUDY OF CHILDREN BORN TO HIV POSITIVE MOTHERS
MULTI-CENTRE EEC STUDY

LABORATORY INVESTIGATIONS

Centre 1-2
Mothers Study Number 3-5
Child Study Number 6

~~Assessment at:~~
~~0-7 days, 3w, 6w, 4.5m, 6m, 9m, 12m, 18m, 24m and then annually if child presumed not infected, or 6 monthly if infected~~

Ring findings and specify as appropriate: R/F/U/N refers to titres **R**ising/**F**alling/**U**nchanged/**N**o previous test

OFFICE USE C

Date blood drawn: _____ / _____ / _____
day month year

HIV/ELISA + / - Specify system used 13-14

Specific antibodies p24 + / - R / F / U / N 15-16
gp41 + / - R / F / U / N 17-18
Other (specify) ..... + / - R / F / U / N 19-20

Western blot + / - R / F / U / N 21-22

Specific antibodies p24 + / - R / F / U / N 23-24
gp41 + / - R / F / U / N 25-26
Other (specify) ..... + / - R / F / U / N 27-28

Virus culture

+ / - Specify identification system(s) 29-30
+ / - Specify identification system(s) 31-32

Antigen assay + / - **R / F / U / N**

Specify identification system 33-35

Other tests (eg IVAP, PCR, IgM) Specify method

+ / - R / F / U / N ..... N/T 36-38
+ / - R / F / U / N 39-41

IgG (gm/litre) 42-44
IgA (gm/litre) 45-47
IgM (gm/litre) 48-50
T4 (10^9/litre)
T8 (10^9/litre)
Absolute lymphocyte (10^9/litre)
Neutrophil (10^9/litre)
Platelet (10^9/litre)
Haemoglobin (gm/dl) 71-73

Toxo IgG Latex (at 9 months to exclude congenital infection) (+/-) 74

Tetanus IgG (at least 1 month after third DT/DPT) 75-77

CMV IgG (+/–) 78

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Treatment of human immunodeficiency virus antibody positive children with intravenous immunoglobulin

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Summary: Five human immunodeficiency virus (HIV) antibody positive children developed recurrent infections requiring multiple hospital admissions. These comprised mainly upper respiratory tract infections, otitis media, pneumonia and diarrhoea, and there was failure to thrive despite adequate antibiotic therapy. They were commenced on iv immunoglobulin (IVIG) therapy and are now relatively free of serious infections and are gaining weight. This therapy was associated with a major reduction in the hospitalization required. In HIV antibody positive children the onset of serious infections, particularly with encapsulated gram-positive organisms, should be taken as an indication for the commencement of regular iv infusions of immunoglobulin.

Keywords: Human immunodeficiency virus; sepsis; paediatric aids; immunotherapy.

Introduction

Infection with human immunodeficiency virus (HIV) in infants may result in antibody as well as cellular immune deficiency (Rubinstein *et al.*, 1983; Bernstein *et al.*, 1985). The main clinical features of this are shown in Table I. Preliminary data in infants with acquired immunodeficiency syndrome (AIDS) or the AIDS-related complex (ARC) suggest that such infants benefit from iv immunoglobulin (IVIG) therapy (Cavelli & Rubinstein, 1986). Of 38 children born to HIV-seropositive mothers in Edinburgh, two suffer from recurrent bacterial infections without typical opportunistic infections. We report the preliminary results of treatment with IVIG in these two children, and in an additional three children treated similarly with Scottish National Blood Transfusion Service IVIG.

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Table I. *Features of antibody deficiency in children with HIV infection*

Clinical features
Recurrent bacterial infections
Recurrent diarrhoea
Resistant thrush
Diffuse lymphadenopathy
Hepatosplenomegaly
Lymphoid interstitial pneumonitis
Immunological features
Diminished in-vivo primary antibody response to T-cell-dependent (tetanus toxoid, KLH, bacteriophage øx 174) and T-cell-independent (pneumococcal) antigens
Depressed or absent amplification of the primary antibody response on secondary challenge with antigen
Depressed or absent IgM to IgG class switch in the secondary response
Poor in-vitro responses to the lymphocyte mitogens PHA and Con A (both primarily T-cell-dependent) with especially poor responses to PWM (B- and T-cell-dependent) and staphylococcal Cowan A protein (T-cell-independent)

Case reports

Patient 1

This patient was born at 42 weeks gestation by spontaneous vaginal delivery after an uneventful pregnancy and weighed 3·25 kg at birth. Her mother had previously abused drugs intravenously and was known to be HIV antibody positive. The infant was monitored regularly as part of a prospective study of perinatal HIV infection.

At the age of 6 months, the patient was admitted to hospital for a week with an upper respiratory tract infection, improving after treatment with amoxycillin. At that time, bilateral axillary lymphadenopathy and a 3-cm palpable liver were noted, and HIV antibody seropositivity confirmed. A month later she was admitted for a fortnight with measles and an inflamed right eardrum. She was treated with hyperimmune measles immunoglobulin ($0{\cdot}1\ ml\ kg^{-1}$) and erythromycin and improved. Bilateral cervical lymphadenopathy was also present.

At 9 months she was failing to thrive and was admitted with a week's history of vomiting (×4/day) and watery diarrhoea (×5/day). Her grandmother had had similar symptoms a week earlier. She was miserable, irritable and had a purulent nasal discharge. The weight was 6·5 kg (below third centile) and she had an inflamed right eardrum. There was bilateral cervical, axillary and inguinal lymphadenopathy and the liver was still 3 cm palpable with no splenomegaly. She failed to improve and after a week developed crackles in both lung bases. Relevant investigations at that time revealed Hb $11{\cdot}2\ g\,l^{-1}$, WCC $13{\cdot}1 \times 10^9\,l^{-1}$, 59% lymphocytes (21·2% T_4 cells, 20·7% T_8 cells, T_4/T_8 ratio of 1·0), normal LFTs, IgG $6{\cdot}6\ g\,l^{-1}$ (3·6–9·6), IgA $1{\cdot}25\ g\,l^{-1}$ (0·16–0·97), IgM 1·35 (0·35–1·40). The chest X-ray showed bilateral perihilar shadowing and the nasal discharge gave a profuse

growth of *Haemophilus influenzae*. No pathogenic organisms were isolated from the stools.

She was treated with erythromycin and weekly IVIG infusions and improved. There was a marked improvement in her mood commencing on the morning following her first infusion, having become alert with spontaneous laughter, and she was discharged after 5 weeks.

The infusion protocol used was 100 mg kg^{-1} for the first two infusions (days 1 and 4) and 200 mg kg^{-1} subsequently (days 7, 14 and 3–4 weekly thereafter). The immunoglobulin preparation was manufactured at the Protein Fractionation Centre, Edinburgh, and was reconstituted as a 4·5% aqueous solution.

In the 13 months since the institution of this treatment she has had three mild upper respiratory tract infections not requiring hospital admission, a PUO and a parainfluenza virus infection both requiring admission, but diarrhoea has not recurred. She has a dry erythematous rash affecting the face and napkin area, which appears 3–4 days before, and clears 1–2 days after each infusion. The lymphadenopathy is unchanged, the liver no longer enlarged, and she currently weighs 9·3 kg (third centile). She is developmentally normal with no abnormal neurological signs.

Patient 2

This patient was born at 38 weeks gestation by forceps delivery after an uneventful pregnancy and weighed 3·33 kg. Her mother denied iv drug abuse. Neonatal jaundice responded successfully to phototherapy and she was discharged after 6 days.

At the age of two months she was admitted to hospital with rotavirus gastroenteritis, and at the age of 10 months was admitted for 12 days with cough, bilateral otitis media and an uncharacterized glandular fever-like illness. Cervical lymphadenopathy, 2-cm hepatomegaly and 6-cm splenomegaly were noted with WCC $50 \times 10^9 l^{-1}$ (12% atypical mononuclear cells) and negative EBV serology. She improved but was again admitted for 3 days a month later because of a troublesome cough.

At the age of 12 months she was admitted with right lower lobe pneumonia, which improved after antibiotic treatment although no organisms were isolated. There were two further admissions over the next 5 months because of a troublesome cough attributed to previous pertussis infection, vaccination having been declined.

HIV seropositivity was first confirmed at the age of 17 months, and was also confirmed in both parents. At that time she was anti-HBc positive but anti-HBs negative; there was lymphadenopathy in the right axilla and both groins. The liver was just palpable but the spleen was not.

At the age of 23 months she continued to have recurrent upper respiratory tract infections and impetigo, not requiring hospital admission. The weight was 12 kg, height 85 cm (both 50th centile). A purulent nasal discharge and peri-oral impetigo were present, with enlargement of the

occipital, left axillary and both inguinal lymph nodes. The liver was palpable at 1 cm and the spleen at 0·5 cm. Investigations showed Hb 11·9 $g\,l^{-1}$, WCC $8{\cdot}9 \times 10^9\,l^{-1}$, with normal differential count, platelets $53 \times 10^9\,l^{-1}$ (rising spontaneously to $108 \times 10^9\,l^{-1}$ a month later), total IgG slighty raised at 14·6 $g\,l^{-1}$ (5·0–13·0), IgA 1·35 $g\,l^{-1}$ and IgM 0·85 $g\,l^{-1}$ (both normal).

She was commenced on IVIG at 24 months and in the 10 months since the initiation of this treatment she has had two episodes of diarrhoea and occasionally a runny nose. She was admitted to hospital once with vomiting, diarrhoea and pyrexia, all of which settled spontaneously with no pathogens isolated. There was an improvement in mood similar to that in Patient 1, but less marked, and she is currently bright and cheerful. She also has a dry macular rash on the face and thighs which appears shortly before and disappears following infusions. She currently weighs 13·7 kg (50th centile).

Patient 3

This patient was born by spontaneous vaginal delivery at term weighing 3·15 kg. He received the usual childhood immunizations and developed normally. At 21 months his mother was admitted with *Pneumocystis carinii* pneumonia, was found to be HIV seropositive, and died. HIV seropositivity was then confirmed in this child and his father, but not in his brother aged 6. The transmission of infection is assumed to have been vertical although the source of infection in the family is uncertain.

He was well until aged 22 months, when he had a pertussis infection requiring hospitalization for 4 days. At that time he had bilateral cervical, axillary and inguinal lymphadenopathy and weighed 10·3 kg (below tenth centile). A month later he had a submandibular swelling of uncertain cause. When this was incised no pus was found and its histology was normal.

At 26 months he had a severe pneumonia. The chest X-ray demonstrated consolidation in the right lower and middle lobes with patchy shadowing in the upper lobe. *Haemophilus influenzae* was isolated from the sputum but he failed to respond to antibiotic treatment until given high dose cotrimoxazole, which produced clinical and radiological resolution. Ten days after being admitted he developed a spastic paraparesis and expressive dysphasia. The serum IgG level was 16 $g\,l^{-1}$, circulating T_4 count $0{\cdot}98 \times 10^9\,l^{-1}$, and T_8 $1{\cdot}3 \times 10^9\,l^{-1}$. The CSF contained a higher anti-HIV antibody titre than did blood, and a CT scan demonstrated cerebral atrophy. Primary HIV encephalopathy was diagnosed and he commenced treatment with IVIG. Two weeks later he was started on AZT (100 $mg\,m^{-2}$ qds by mouth). He was discharged 10 days later.

There has been considerable improvement during the 3 months of follow-up. He is now able to run and there is improvement in the dysphasia. The inguinal nodes are palpable, the liver 2 cm enlarged, and he weighs 12·35 kg (above tenth centile). The CT scan is normal and the T_4 and T_8 numbers are $2{\cdot}48 \times 10^9\,l^{-1}$ and $0{\cdot}68 \times 10^9\,l^{-1}$ respectively. The serum IgG

concentration is 18·9 g l^{-1} (6–16), IgA 1·9 (0·65–3·0) and IgM 1·78 (0·25–2·0).

Patient 4

This patient was born by spontaneous vaginal delivery at 43 weeks gestation weighing 3·15 kg. She was well until aged 28 months, when she was admitted for exploration of both groins as she was thought to have herniae. Inguinal lymphadenopathy only was found, which later resolved, and the lymph node biopsy showed reactive hyperplasia. She had whooping cough at 30 months and was admitted for 4 days at 33 months with a urinary tract infection.

At the age of 44 months there was a marked deterioration in her health, with recurrent right-sided pneumonia necessitating four hospital admissions lasting 8, 7, 7 and 38 days respectively, the sputum yielding *Mycoplasma pneumoniae* and *Haemophilus influenzae* on the first and last admissions respectively.

During the last admissions she weighed 10·1 kg (below third centile, age 46 months) and HIV seropositivity was confirmed. Both parents had previously abused drugs and were also found to be HIV seropositive. She was started on azothymidine (AZT) 100 mg m^{-2} qds by mouth, regular antibiotics and IVIG.

During the 3 months of follow-up her weight has increased to 12 kg (still below third centile) and she has been admitted once for 3 days on account of a *Haemophilus influenzae* chest infection. AZT was stopped a week after this admission because of leucopenia (WCC $2{\cdot}9 \times 10^9\,l^{-1}$).

Patient 5

This patient underwent corrective surgery for a congenital cardiac lesion aged 4 months, when he was transfused blood from a donor later found to be HIV seropositive (prior to HIV antibody screening of all blood donations). His cardiovascular progress was satisfactory and he was otherwise well apart from low weight (below third centile).

From the age of 14 months he had frequent respiratory tract infections and tonsillitis, one of which required hospital admission.

At 21 months he presented with a petechial rash. The liver was palpable at 3 cm as was the tip of the spleen, and the platelet count was $< 10 \times 10^9\,l^{-1}$ with normal/increased megakaryocytes in the bone marrow. The serum IgG concentration was 29 g l^{-1}. He was commenced on prenisolone 2 mg kg^{-1} day^{-1} for a week, later reducing to 5 mg day^{-1}, but platelet counts did not rise. Over the following 3 months he received three courses of high-dose IVIG (2 g kg^{-1}), which produced transient increments only in platelet counts. These were to $62 \times 10^9\,l^{-1}$ on day 3 of the first course, falling to < 10 by day 7; to $190 \times 10^9\,l^{-1}$ on day 5 of the second course, falling to < 15 by day 14; to $120 \times 10^9\,l^{-1}$ on day 3 of the third course, falling to < 11 by day 14. Whilst on prednisolone 5 mg on alternate days, aged 25 months,

the platelet count rose to $59 \times 10^9 l^{-1}$ and has been between 20 and $139 \times 10^9 l^{-1}$ since.

When aged 26 months he had otitis media and gastroenteritis. Over the next 15 months he had two mild upper respiratory tract infections and a further three episodes of otitis media, causing perforation of the right eardrum.

At 42 months he was admitted with pyrexia, diarrhoea and vomiting which settled after treatment with iv cotrimoxazole. Bilateral cervical lymphadenopathy was present and the liver was palpable at 2 cm, the spleen at 1 cm. HIV seropositivity was confirmed. The oldest library serum sample available, taken aged 21 months, also showed HIV seropositivity.

He was started on regular low-dose cotrimoxazole (40 mg bd) and IVIG and in the 2 months of follow-up has been free of infections. Pre-infusion serum IgG levels remain very high (currently $>46\ g\ l^{-1}$).

Discussion

The usual clinical presentation in infants, with recurrent bacterial infections, is very similar to that of patients suffering from primary hypogammaglobulinaemia. Despite the polyclonal increase in immunoglobulin concentrations, the very poor primary and secondary specific responses to antigen challenge (Table I) (Rubinstein *et al.*, 1983) render such infants susceptible to bacterial infection. Clinical benefit may result from the administration of normal immunoglobulin by virtue of its content of antibodies whose specificities may be more appropriate to any prevailing infection.

In view of the clinical and immunological similarities to hypogammaglobulinaemia, IVIG has been used in the treatment of infants with AIDS. Some of the immunological abnormalities associated with AIDS are reversed by treatment with IgG. Peripheral blood T cells from infants with AIDS are unable to suppress Pokeweed Mitogen (PWM)-driven IgG secretion from normal peripheral blood lymphocytes. Following treatment with IVIG for 4–13 months this function returns to normal (Gupta, Novick & Rubinstein, 1986). In one series of 31 children with AIDS there was a reduction in the incidence of septicaemia from 18/27 in non-IgG-treated controls to 1/14 in IgG-treated infants associated with such therapy (Calvelli & Rubinstein, 1986).

Pre-infusion IgG levels are rather difficult to interpret at this stage in the treatment of these children, as there is a background of polyclonal increase in IgG concentrations. Consequently, they have not yet been used to modify the regime of treatment given, which is similar to that used initially in the antibody replacement therapy of patients who suffer from primary hypogammaglobulinaemia (Leen, Yap & McClelland, 1986).

The point at which one should initiate treatment with IVIG in such children is a matter for debate. We would suggest that after two or

Table II. *Clinical features of five children with HIV infection treated with IVIG*

	1	2	3	4	5
Sex	F	F	M	F	M
Present age (months)	22	34	29	49	44
Lymphadenopathy	Y	Y	Y	—	Y
Enlarged liver/spleen	Liver	Both	Liver	—	Both
Encephalopathy	—	—	Y	—	—
Thrombocytopenia	—	—	—	—	Y
Otitis media	×2	×1	—	—	×4
Pulmonary infections	1	2	2	5	1
Days in hospital in 12 months before IVIG therapy	53	26	34	60	7
Months of IVIG therapy	13	10	3	3	2
On AZT	—	—	Y	Y*	—
On regular antibiotics	—	—	—	Y	Y
Days in hospital since IVIG therapy started	20	6	0	3	0

*Stopped after 1 month because of leucopenia.

more serious infections occur, involving infection with encapsulated gram-positive organisms, then long-term treatment with IVIG is probably indicated.

Regular IVIG therapy did not prevent the occurrence of infections during the period of follow-up (2–13 months, Table II), but infections while receiving this treatment were less severe, as borne out by the major reduction in the duration of hospitalization. Consideration should therefore be given to starting therapy in asymptomatic HIV antibody positive children since it is possible that intercurrent infection with organisms other than HIV may be a co-factor in the progression of the HIV infection. This could perhaps occur by infection causing T_4 lymphocyte activation, including that of latently HIV infected cells, with consequent viral replication and acceleration of disease progression.

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MOTHER-TO-CHILD TRANSMISSION OF HIV INFECTION

THE EUROPEAN COLLABORATIVE STUDY*

Summary 271 children born to HIV-infected mothers in 8 European centres are being followed up from birth in a multicentre, collaborative study. By June, 1988, 45% had been followed for over 1 year: 10 had developed AIDS or AIDS-related complex, all by the age of 9 months, of whom 5 had died. 22 other children had symptoms or signs suggestive of HIV infection; of these, 12 had immunological abnormalities, 9 of whom were infected. 5 children had problems not related to HIV, including 3 neonatal deaths. The other 234 children are immunologically normal and clinically well. The median age of antibody loss was 10·3 months, although 1 did not lose antibody until over 18 months. None lost antibody and then became and remained seropositive. Of 100 children followed for more than 15 months, 19 had persistent antibody, and 5 were antibody-negative but presumed to be infected because of virus isolation or antigen detection; these 5 children were clinically and immunologically normal. The estimated vertical transmission rate was 24%.

Introduction

BY June 30, 1988, 1528 women and 356 children under 13 years of age had been reported to the WHO European AIDS surveillance centre. Half the women were intravenous drug abusers of child-bearing age; three-quarters of the children had acquired their infection from a mother who either had AIDS or who was at risk of AIDS.[1] Since the number of women with human immunodeficiency virus (HIV) infection greatly exceeds those with AIDS, these figures considerably underestimate the number of children who may be at risk.

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The natural history of HIV infection in haemophiliac children has been well investigated;[2] much less is known about the risk or long-term outcome of perinatal infection. Reported rates of infection for children born to HIV-seropositive mothers range from 0 to 65%.[3] However, some studies are based either on prospective follow-up of children who already have symptoms or whose mothers have had a child with AIDS; others are based on small numbers or short follow-up.[4-9]

The prognosis for children with AIDS is poor,[10] but little is known about outcome for the much larger proportion of HIV-infected children with less severe or no symptoms. We report results from the first 271 children enrolled in a multicentre European study,[11] which was set up to determine rates of transmission of HIV infection from a seropositive mother to her infant; to assess factors that might influence transmission such as mode of delivery, breastfeeding, and mother's clinical status during pregnancy; and to determine the natural history of perinatal HIV infection.

Subjects and Methods

HIV-seropositive women are systematically identified in the antenatal period at all the participating centres. Children are included only if the paediatrician is aware of the mother's seropositive status before or at the time of birth; all such children are included. One paediatrician in each centre coordinates the follow-up. Children are examined shortly after birth and at 3-monthly intervals. Information gathered at birth includes details of maternal history (eg, symptoms in pregnancy, date of HIV test, likely route of transmission, marital status, age, and parity). Perinatal details include mode of delivery, birthweight, gestational age, the presence of congenital abnormalities or drug withdrawal symptoms, and any abnormal clinical findings. At subsequent examinations, the last date of breastfeeding is recorded, with an interval history and details of immunisations; a physical and developmental examination is done and height, weight, and head circumference are measured. Particular attention is paid to the presence of lymphadenopathy, hepatosplenomegaly, persistent oral candida, skin infections, non-infectious skin eruptions, confirmed bacterial sepsis, persistent or recurrent diarrhoea, persistent fever, radiologically confirmed pneumonia or pneumonitis, developmental delay, and abnormal neurological findings. A social history is obtained with particular reference to the child's carer and the health of the mother.

A blood sample is taken at each visit and HIV antibody is measured by enzyme-linked immunosorbent assay (ELISA) and western blot. Virus culture and antigen tests are requested every 6 months. An in vitro test of IgG antibody production[12,13] is done in Padua and Genoa. Other investigations include absolute lymphocyte count, platelet count, immunoglobulin subsets, and a

TABLE I—CLINICAL HISTORY OF CHILDREN WHO HAD AIDS OR ARC

Sex	Birth-weight (kg)	Gestational age (wk)	Major clinical manifestations in order of appearance	AIDS/ARC onset (wk)	Age last seen (wk)	Virus ever	Antigen	T4 (at last visit)	T8	IgG†
M	3·22	40	POC; L; HS; IP; LDM; Enc; DCMV	18	28*	−	−	0·2	2·3	18·8
M	3·50	40	L; HS; POC (resolved)	9	108	+	−	1·2	2·4	25·1
F	2·50	39	HS; L; POC; DCMV	9	12*	+	+	4·5	4·1	9·7
F	1·84	37	L; HS; POC; LDM; FTT; acquired microcephaly; spastic quadriplegia; PCP	34	86*	+	+	0·2	0·3	1·5
M	3·04	40	L; HS; motor delay; hypertonus; POC (resolved); PS	37	86	+	−	1·3	3·4	8·6
M	3·34	40	POC; L; HS; lymphoid interstitial pneumonitis	17	19*	+	+	1·6	1·2	3·6
M	3·16	37	POC; L; D; SID	24	28*	ND	ND	4·1	1·3	2·8
M	2·90	39	POC; L; HS; IP; PS	12	25	+	+	1·0	0·6	21·3
F	3·27	40	D (resolved); L; HS; pneumonia; FTT	36	117	+	−	0·6	1·6	6·6
F	2·65	41	POC; L;HS; PCP; FTT	24	53	ND	+	1·0	0·5	10·2

*Died; †IgG at last visit before treatment.
POC = persistent oral candida; L = lymphadenopathy; HS = hepatosplenomegaly; IP = interstitial pneumonitis; LDM = loss of developmental milestones; Enc = encephalopathy; DCMV = disseminated CMV; FTT = failure to thrive; PCP = *Pneumocystis carinii* pneumonia; PS = parotid swelling; D = chronic diarrhoea; SID = sudden infant death; ND = not done.

measure of B-cell function in response to diphtheria and tetanus immunisation. Urine is cultured for cytomegalovirus (CMV) shortly after birth and blood is tested serially for toxoplasmosis IgG. Virus cultures and antigen tests are done as previously described.[11] Positive virus cultures are confirmed by antigen capture. Centres differ in the frequency of virus and antigen tests and not all centres performed both tests.

Definitions of Infection

Infection was defined by persistence of antibody (as measured by ELISA, confirmed by western blot) beyond the age of 15 months, clinical AIDS or AIDS-related complex (ARC), or the presence of virus or p24 antigen. Children who lost antibody and did not have a positive virus culture or antigen test were presumed not to be infected. Maternal antibody may persist well into the second year of life therefore, in the absence of virus isolation or antigen detection, the infection status of antibody-positive children under 15 months old was considered indeterminate.

AIDS was defined according to Centers for Disease Control (CDC) surveillance criteria.[14] A child was considered to have AIDS-related complex (ARC) if interstitial pneumonitis, persistent oral candida, or progressive encephalopathy had been present for at least 2 months, with two or more of: persistent general lymphadenopathy, hepatomegaly or splenomegaly, chronic or recurrent episodes of diarrhoea, failure to thrive, or parotid swelling. All such children would be included in class P2 of the CDC classification system for HIV infection in childhood.[15] The date of onset of AIDS or ARC was taken as the date of the first clinical examination when this definition was met.

Results

Maternal Characteristics

By the end of June, 1988, 271 children born to 264 mothers were enrolled including 3 pairs of twins and 4 second births. Compliance with follow-up has been good: only 8 of 147 children under 18 months have not been seen for over 6 months, and 11 of 116 children over 18 months have not been seen for over 12 months. The age of mothers ranged from 14 to 38 years (median 24·7); 67% of mothers were married or cohabiting; and for 65% this was their first child. All but 12 were caucasians. 85% of mothers, and a further 7% of partners, had a history of intravenous drug abuse, and only 3% had no risk factor identified. 14 mothers (5%) had AIDS or ARC before delivery.

Perinatal Outcome

Mean birthweight was 2·82 kg (range 0·79–4·48) and mean gestation 38 weeks (range 25–43); 142 were boys, 129 girls. Drug-withdrawal symptoms were present in 68 (25%) but, apart from problems related to prematurity, perinatal findings were unremarkable. Congenital abnormalities were noted in 7: Poland's syndrome, polydactyly, syndactyly, cleft palate, polycystic kidneys, hydronephrosis from pyeloureterostenosis, and cystic fibrosis. Another child had cerebral palsy and a ventriculoperitoneal shunt sited because of neonatal meningitis. 2 infants died in the first 48 hours, both of birth asphyxia: one was a girl of 790 g born at 25 weeks' gestation, the other a 2440 g boy delivered by emergency caesarean section at 37 weeks' gestation; a third child who weighed 1225 g at birth after 33 weeks' gestation died of respiratory distress syndrome and intracranial bleeding after 20 days. These 3 deaths are presumed not to be HIV-related.

Birthweight standard deviation (SD) scores (birthweight standardised for gestational age and sex) were derived from the most appropriate British and North American standards.[16,17] HIV infection was not related to gestational age, to birthweight, or to birthweight SD scores. Birthweight SD scores were below zero in all centres, (overall mean −0·84 SD units), and were related to mothers' use of drugs and to centre, with differences between Spanish (mean −1·10 SD units), Italian (−0·61), and north European (−0·90) centres. When these differences between centres were allowed for by multiple regression, differences were found between the birthweight SD scores of infants born to mothers who did not use drugs in pregnancy (SD score −0·70) and infants with drug withdrawal symptoms (−1·08). Infants whose mothers had used drugs in pregnancy but had no withdrawal symptoms had an intermediate mean birthweight SD (−0·87). This was a statistically significant linear trend ($t_{248} = 2·15$, $p = 0·032$).

Clinical Follow-up

178 of 268 children who survived the neonatal period have been followed up for at least 6 months and 123 for over 1 year. Table I shows the 10 children who had developed AIDS or ARC, of whom 5 have died: 4 with opportunistic infections, and 1 recorded as a sudden infant death. All the children with AIDS or ARC were antibody-positive, 8 out of 9 tested were virus-positive, but not all had immunological abnormalities (table I). The age of onset of AIDS or ARC is shown in fig 1 which includes only the 34 children known to be infected. It shows the age when last seen, or the age at onset of AIDS or ARC. 29% of infected children have developed AIDS or ARC, all by the age of 9 months.

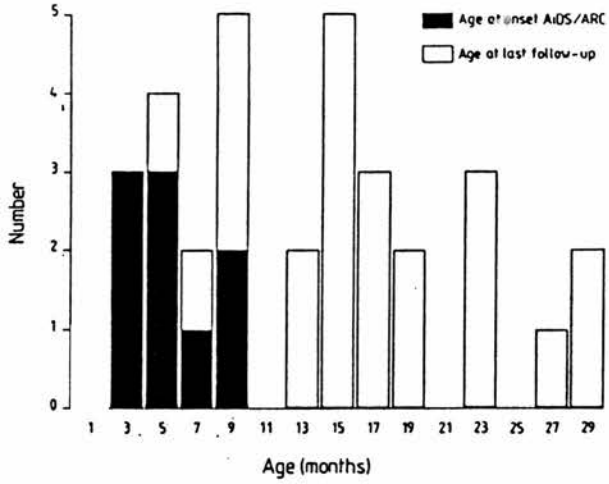

Fig 1—Age at onset of AIDS or ARC or age at last follow up of 34 infected children.

22 other children have non-specific signs or symptoms of HIV infection, assessed solely on the basis of clinical examination. (Findings included: persistent generalised lymphadenopathy, persistent hepatomegaly or splenomegaly, chronic diarrhoea, acquired microcephaly, growth retardation, or regression of developmental milestones.) 9 of these children are presumed infected and all have low T4:T8 ratios (< 1·0) or hypergammaglobulinaemia, compared with 1 of 5 presumed not infected and 2 of 8 indeterminate. Some children previously in this category have had total resolution of their symptoms. The only child with neurological abnormalities—epilepsy and acquired microcephaly—was antibody-negative at 63 weeks and presumed not to be infected. Apart from 1 child with cystic fibrosis and 1 with cerebral palsy, the remaining 234 children were clinically well and had no signs of neurological damage or developmental delay at the time of their last visit. There were no cases of AIDS dysmorphic syndrome[18] and no immunological abnormalities in this group.

HIV Antibody Status

Fig 2 shows the estimated cumulative percentage to lose antibody at each age. (Age at antibody loss was taken to be midway between the last positive and the first negative test.) Children who died of AIDS or ARC, all of whom were antibody-positive when last seen, are assumed to have had persistent antibody. The median age at antibody loss, among those who lost antibody, was 10·3 months. 53% lose antibody by 1 year (95% confidence interval 45%–62%). By 15 months, 69% would be expected to have lost antibody (95% CI 61%–77%). However, of the 107 seronegative children, 6 have lost antibody after 15 months; one had been antibody-positive at 18·1 months. No child has lost antibody and then become, and remained, antibody-positive; 20 children have been observed for at least one year after they became seronegative.

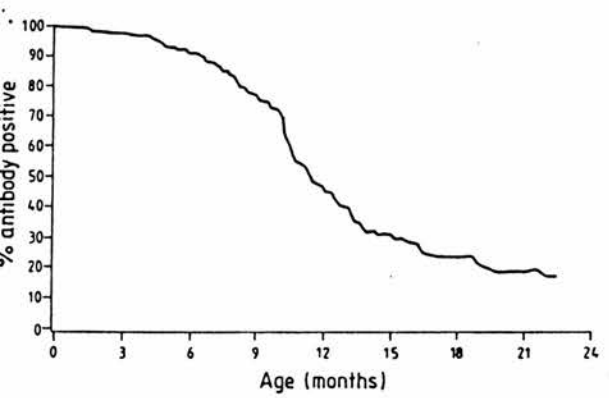

Fig 2—Loss of antibody.

TABLE II—CLINICAL AND INFECTION STATUS OF CHILDREN OVER 15 MONTHS

	AIDS/ARC	Symptoms suggestive of HIV	Well	Total
Presumed infected				
Persistent antibody	8*	8	3	19
Antibody negative, virus/antigen positive	0	0	5	5
Presumed not infected				
Antibody negative	0	4	72	76
Total	8	12	80	100

*Includes 4 children who died with AIDS/ARC but who would have been over 15 months at the time of analysis.

Transmission of Infection

Only the 96 children who were older than 15 months when last tested were included to establish the transmission rate for perinatal infection. 15 (16%) of this group remained antibody-positive and are therefore presumed infected, and an additional 4 children died of AIDS or ARC but would have been over 15 months had they survived. Of the 81 who were antibody-negative, 5 (6·2%) are considered infected because of at least 1 virus isolation or positive antigen test. This is probably an underestimate of the prevalence of infection in the antibody-negative group, as these tests are insensitive and not done at every follow-up. A minimum estimate of the transmission rate was therefore 24% (95% CI 16–32%).

Clinical findings were related to presumed infection status (table II). Of the 76 antibody-negative children surviving to 15 months, 4 (5%) have HIV-related symptoms, compared with 8 of 11 (73%) with persistent antibody who have not shown AIDS or ARC. None of the 5 antibody-negative but presumed infected children had HIV-related symptoms. (The difference between the two presumed infected groups in the frequency of HIV-related symptoms is significant; $p = 0{\cdot}026$, Fisher's exact test, 2-tailed.)

Risk Factors

We found no evidence that mother's clinical status at delivery, mode of delivery, or breastfeeding[19,20] influenced transmission rates, but the small sample size and other confounding variables made accurate risk estimates impossible.

Discussion

The management of HIV infection in infants is complicated by the difficulty of early diagnosis. At first it was believed that maternal antibody would disappear by 6 to 12 months, but with antibody loss reported at ages of 15 months or even later,[15] the definition of infection was revised to include only those children in whom antibody was

present after 15 months. In our study, 6 children who lost antibody did so after 15 months. Several children have been reported to lose antibody only for it to reappear at 20 to 24 months,[21] but in our study no children have yet lost antibody and then become, and remained, antibody-positive. All chilđen with AIDS or ARC have been antibody-positive. In children, loss of antibody may not prove absence of infection: children have been reported with negative ELISA and western blot, but have been positive on virus or antigen testing and presumed, therefore, to be infected.[22,23] These were all children with symptoms and evidence of immunosuppression. By contrast, in this study, the 5 antibody-negative children found to be positive on virus or antigen tests were immunologically normal and without clinical symptoms at their last assessment; 4 have been followed up for periods of between 5 and 12 months since they first became seronegative. This observation is not consistent with the view that antibody is lost as the children become ill, and requires confirmation from prospective studies. New laboratory tests such as the in vitro test of IgG antibody production,[12,13] the in situ hybridisation technique described by Harnish,[24] and western blot analysis of the antibody profiles of mother and child[25] may facilitate the early diagnosis of HIV infection in children. The polymerase chain reaction technique[26] shows promise, but will need careful evaluation of its sensitivity and specificity.

We found an overall transmission rate of 24% for perinatally acquired HIV infection, but this is probably an underestimate because of the insensitivity of virus and antigen tests. Higher rates based on antibody status at less than 15 months have been reported,[5,9] but these will tend to be overestimates: for example, had antibody presence after 1 year been used to define infection in our study, the transmission rate would have been 53% not 24%. Case selection may also account for differences in reported transmission rates: in the early stages of the study data were forwarded to us on children who had not been identified in the first 3 weeks of life and did not fulfil the study criteria. Most of this group had HIV-related symptoms and were infected.

Our preliminary report[11] suggested an association between clinical symptoms in the mother in the antenatal period and the early onset of AIDS or ARC in infants. However, no association was apparent with more data and the use of HIV infection rather than AIDS or ARC as the measure of outcome. Most children in the European study were born to mothers who abused intravenous drugs; as well as exposure to intrauterine HIV infection, these infants were at increased risk of other congenital infections, low birthweight, neurological disorders resulting from drug withdrawal, and other perinatal problems. The observed excess perinatal mortality is probably due to the background of drug abuse—as demonstrated by the association of low birthweight and drug abuse during pregnancy—rather than an effect of HIV infection alone. In many, social deprivation was encountered after birth, which could have adversely affected the child's development and health.

Most of the 271 children born to HIV-positive mothers in the European study remain clinically well. However, for the 4 children over 15 months of age who are presumed to be infected the outcome is poor (table II): 8 had acquired AIDS or ARC, 5 of whom had died and only 8 (33%) remain without symptoms. Neurological problems occurred in 3 of the AIDS or ARC children; they were present in only 1 child who was presumed not infected. Neurological manifestations of HIV infection may become evident as the children are followed for longer periods.[27] Like other investigators,[18,28] we are unable to confirm an AIDS dysmorphic syndrome. The importance of not labelling children as infected on the basis of signs less severe than AIDS or ARC was confirmed by the resolution in some children of nonspecific HIV-related findings, with loss of antibody. However, nonspecific symptoms or signs with low T4:T8 ratios and hypergammaglobulinaemia strongly suggested infection. Continued follow-up of children who become antibody-negative is essential since it still cannot be assumed that they have escaped infection, even if they are clinically well.

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The management of children born to human immunodeficiency virus seropositive women

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Summary

Increasing numbers of children born to human immunodeficiency virus (HIV) antibody-positive women are being identified, but guidelines as to their management are lacking. We have therefore established a paediatric counselling and screening clinic for managing such children in Edinburgh. During a period of 3 years, 49 infants and children of 43 HIV seropositive women have been seen. After a median follow-up period of 23 months, four children were found to have clinical evidence of HIV disease which was non-specific and could have been missed had they not been regularly monitored. Thus, close surveillance of infants born to seropositive women is important. Identifying a single clinic where this is done has allowed experience to accumulate on issues beyond the medical management of these infants as well as contributing to the clinical care of infants with symptoms. Based on this experience, we have developed guidelines for managing children born to HIV antibody-positive women.

Introduction

The human immunodeficiency virus (HIV) is now known to be infecting increasing numbers of women, many of whom are young and sexually active. Therefore obstetric and perinatal issues need to be considered.[1] Debate continues as to whether termination of pregnancy should be advocated for all pregnant women who are seropositive for HIV because of risks to the mother and child. Little is known about the natural history of perinatally acquired HIV infection, nor do guidelines exist for managing children born to women who are infected with HIV.

In Scotland, studies of intravenous (IV) drug abusers have been shown that between 38 and 54% are infected with HIV.[2–5] We therefore set out to identify women who were HIV antibody-positive and who had recently had infants or who were in various stages of pregnancy. On 1 January 1986, a clinic was established to provide paediatric advice and to monitor the progress of these infants. This report describes the experience during the first 33 months and the guidelines for management that we have developed.

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Methods

Paediatric counselling and screening clinic

The clinic is staffed by a consultant paediatrician with an interest in community child health (JM). It is held in conjunction with an adult counselling and screening clinic supervised by a consultant in infectious diseases (RPB). It operates with two sessions a week, during which a health visitor is present and, at one of the sessions, a dental hygienist also. Referrals are made by staff in the adult clinic once a pregnant woman is identified. Other sources include staff in antenatal clinics, neonatal paediatricians, general practitioners, social workers, and health visitors upon discovery of a woman and infant considered to be at risk of HIV infection. Latterly, with the introduction of a research registrar, the women have been seen in antenatal clinics where the purpose and nature of the surveillance is explained, after which maternal consent is sought. The research registrar has also been present at delivery so as to collect cord blood, to examine the infant at birth and to arrange future visits for the mother, either in the clinic or at home according to her preference.

Surveillance procedure

The procedure adopted is summarised in Table I. Infant and mother are seen at 6 weeks, 3 months, and 3-monthly thereafter. At each visit, a clinical history is taken to elicit symptoms of HIV disease. The health visitor assesses development by means of the Denver Developmental Screening Test, after which the paediatrician examines the infant. Measurements made include weight, head circumference and length (or height) of the infant. Peripheral blood is taken for virology (HIV antibody, antigen and virus culture), immunology (immunoglobulins, T-lymphocyte subsets) as well as haematology (full blood count with differential and platelet count).

Immunisation procedures are discussed and, due to difficulties at other clinics in obtaining inactivated polio vaccines, the children are usually immunised by us. Other child care issues are raised, and the child's mother has the option of seeing an adult physician at the same appointment, if she so wishes. Families seen at home are given a separate appointment when a physician visits the mother.

Further management

When an infant manifests signs or symptoms of HIV or other infection, in-patient facilities are available at the infectious diseases unit of the City Hospital, Edinburgh where admission is under the care of the consultant paediatrician. Recurrent bacterial infections are seen in children infected with HIV, despite hypergammaglobulinaemia.[6] We have instituted regular IV infusions of 200 mg/kg body weight of immunoglobulin (IV IgG obtained from the Scottish National Blood Transfusion Service) every 3 weeks to symptomatic children who present with recurrent bacterial sepsis.[7] When, despite IV IgG, the absolute T4 cell count has declined or when HIV antigenaemia and loss of core antibody have persisted, zidovudine (14 mg/kg per day) has been given orally.

Table I *Guidelines for managing children born to HIV antibody-positive mothers*

At 3 monthly intervals.
Clinical review, particularly for infections
Developmental screening
Laboratory investigations:
Virology (HIV antibody and antigen status)
Immunology (T4 and T8 lymphocyte counts and immunoglobulin values)
Haematology (full blood counts including platelets)
Immunisation
When necessary, liaison with
Adult physician regarding the mother's health
Social work staff on housing and financial matters or alternative arrangements for care of the child
Educational staff (at parent's request)
Physiotherapist, dietitian
Self help groups for emotional support

Table II *Clinical and laboratory evidence of HIV infection in four children*

Persistent lymphadenopathy	4
Hepatosplenomegaly	4
Recurrent respiratory infections	4
Eczematous rashes	3
Purpura	2
Positive HIV culture	4
Persistent HIV antibody	4
Positive HIV antigen test	4
Hypergammaglobulinaemia	4
Thrombocytopenia ($< 150 \times 10^9$/l)	4
Absolute T4 lymphocytopenia ($<$ 1000/l)	4

The chaotic lifestyles led by some parents who continue to abuse drugs have meant that their children have to be taken into care. This has required close liaison with the Lothian Region Social Work Department whose staff have established guidelines for placing these children in alternative care.[8]

Results

From 1 January 1986 to 30 September 1988, 49 infants (age range 1–53 months) born to 43 HIV seropositive women have been seen. Of the 43 mothers, 37 were infected through IV drug abuse, and six because of heterosexual contact. The mothers' age at delivery of the index infant ranged from 17 to 33 years (mean 24·1 years). For 22 mothers, this was the first child, two having had a further child following the birth of the index case. Only 14 continued to use IV drugs during pregnancy but at least 20 have misused drugs since.

During the follow-up period, nine infants were taken into foster care (with

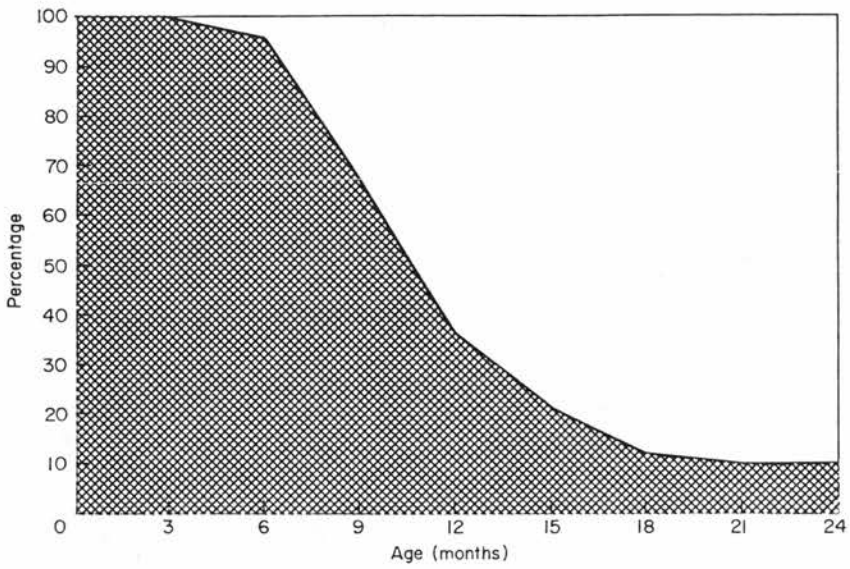

Fig. 1. HIV antibody positive children as a percentage of total tested.

four subsequent adoptions), three were in the care of grandparents and the rest were living with their mothers. Fifteen mothers were single. Some of those with a partner had unstable relationships in which the male partner was often in prison or had left home. Nineteen of the infants were known to have fathers who were themselves HIV seropositive. After a median follow-up of 23 months, four children showed clinical evidence of HIV infection. Signs and symptoms appeared at 6–9 months and are detailed in Table II. In these four children, the clinical evidence for HIV disease was non-specific and could have been missed had they not been regularly monitored. In addition, these four children had recurrent infections which benefited from IV IgG therapy in terms of reduction in episodes of respiratory sepsis and diarrhoea. They also gained weight and spent fewer days in hospital.[7] Two of these children have been given zidovudine orally for nine months without any adverse effects.

Of 37 infants over 15 months of age, four remain HIV antibody-positive. Figure 1 shows the percentage of infants HIV antibody-positive at various ages. The median age at loss of maternal HIV antibody was 12 months (range 6–18). The remaining 45 children (age range 1–53 months) were clinically well when last seen and the developmental progress of the cohort as a whole has been within normal limits.

Of the 49 children, 47 received diphtheria/tetanus (DT) or diphtheria/tetanus/pertussis (DPT) immunisation, 37 inactivated polio vaccine, ten oral polio vaccine, while measles vaccine was given to 20 children over 15 months of age who were HIV antibody-negative, clinically well and had normal tests of immune function. An adverse reaction was not reported in any child or family member.

Discussion

delines for managing children born to HIV antibody-positive women need e based upon adequate data. Prospective studies are therefore needed to blish the prevalence of perinatal transmission as well as the natural history IIV disease in infants.[9] The guidelines that we propose reflect our clinical laboratory data. In establishing that which we have described, a diatrician was appointed to co-ordinate the surveillance of all the children. nburgh has the largest cohort in the U.K. of such infants. This reflects, in , the needle-sharing habits of drug abusers but is also due to there being ngle centre for referrals and where expertise is accumulated. Close itoring of these infants leads to detection of early signs of HIV disease (as ur four HIV-infected children), and therapy such as regular infusions of mmunoglobulin and oral zidovudine. Good rapport with the mothers has meant that no child has been lost to surveillance.

t the time of writing, routine antenatal screening for HIV antibody is not ctised in Edinburgh. However, most women at the time of booking are d about high risk activities and are offered the option of testing. Women are HIV-seropositive and who wish to continue the pregnancy are rred to the paediatricians. The number of infants attending the clinic is bably less than the actual number of infants at risk of HIV infection in the nburgh area. Even so, due to close liaison with obstetricians, paediatricians, sicians and social workers in Edinburgh, the degree of under representation robably small.

uidelines, as well as the rationale, for immunising children infected with have been published.[10] Lack of adverse reactions to live virus vaccines in er the children or other family members supports previous experience. lso stresses the importance of separating HIV-infected children with ptoms from those without symptoms when considering immunisation. risks of natural measles for unvaccinated HIV-infected children who are cally and immunologically healthy far outweigh the theoretical risks of sles vaccination.

ccording to current recommendations,[11] HIV-seropositive women should r pregnancy and termination should be offered to those who are pregnant. ur experience, such sweeping statements deter women from seeking early natal care so that most present too advanced in pregnancy for termination e considered. At our clinic, individual advice is given to each woman templating pregnancy and she is strongly urged to have a thorough medical laboratory evaluation. Pregnancy is contra-indicated if signs of HIV ase are detected or if immunological function is abnormal.

lost seropositive women in Edinburgh have been infected by abusing gs. They come from areas of the city with problems of multiple ivation.[5] Therefore, the problems of drug abuse, unemployment, risonment, and single parent families compound the diagnosis of HIV ction. Parental skills are lacking so that close liaison between doctors and al workers is essential. The paediatric clinic has also served as a resource re for social workers as well as for other health care professionals, self-help ips and members of the public. Information on the risks of perinatal

transmission and on the care of infants considered to be at risk is scanty so that we have had to prepare guidelines for carers. Also, we have identified the social isolation and anxiety of mothers when facing the uncertain future for their children and for themselves. Attempts have been made therefore to start a self-help group for HIV-seropositive mothers. We also envisage that due to clinical deterioration in a mother's health, it may be difficult for her to care for her infant, so we intend to start a supported accommodation unit for mothers and infants.

The mobility of IV drug abusers has recently been appreciated.[6] With reports that suggest a rise in seroprevalence rates in England and Wales,[12] other cities in the U.K. will experience problems with infants born to seropositive women as Edinburgh has done. Costs of the paediatric clinic relate only to the salaries of medical and nursing staff (£23,000 per annum). Other expenses are borne by the adult counselling and screening clinic.[6] At a time of limited resources, the establishment of a single centre in a region for the referral, review and management of infants at risk of transmission of HIV from the mother appears to be the most efficient approach to this difficult problem.

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Vertical transmission of HIV: a prospective study

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SUMMARY Forty nine infants of HIV seropositive women were followed up for a median of 24 months, together with 24 controls. The infection status of 11 index children under 18 months of age was indeterminate; 34 were presumed uninfected while four showed clinical and laboratory evidence of HIV disease. Based on current definitions of HIV infection and excluding children under 18 months old as well as those who had not been studied from birth, two out of 28 children were infected. The estimated rate of maternofetal transmission was therefore 7·1%. In children with proved infection, sequential laboratory data showed that hypergammaglobulinaemia was noted as early as 6 months and often predated clinical signs. This observation, in the presence of non-specific clinical findings, was helpful in alerting the paediatrician to a diagnosis of HIV infection.

Vertical transmission of the human immunodeficiency virus (HIV) is now well documented,[1–3] although the exact risk has not yet been quantified. Rates of transmission appear to vary from 22–65%, depending on inclusion criteria and duration of the study as well as methods used to define paediatric HIV infection.[4–6] The diagnostic value of an HIV antibody test is limited by the presence of passively acquired maternal antibody in infants. Clinical evidence of infection is also unreliable, especially in the early stages when signs and symptoms are non-specific.[7] The inclusion of control children is therefore essential, in order that clinical and laboratory parameters can be evaluated. So far, no study of mother to child transmission of HIV has included a control group.

In the United Kingdom, Edinburgh has been reported to have the largest population of intravenous drug users infected with HIV, one third of whom are young women.[8] Most were infected during an epidemic of needle sharing in 1983–4, and most are asymptomatic at present. Centres which reported high rates of vertical transmission of HIV had initially concentrated on women who had more advanced disease. We postulated that the same risk might not apply when the mother had preclinical disease. Infants born to HIV seropositive women were therefore enrolled into a prospective study to evaluate the risk of maternofetal transmission and to define the natural history of perinatally acquired HIV disease in those who were infected.

Subjects and methods

SUBJECT SELECTION

The paediatric counselling and screening clinic at the City Hospital in Edinburgh was established in January 1986 to monitor all infants born to HIV seropositive women. Edinburgh is one of the centres collaborating in the European Multicentre Study on perinatal transmission of HIV.

Most HIV infected pregnant women were identified in the antenatal period. If the woman chose to continue with her pregnancy, permission was sought for inclusion of her infant in the study. Thirty nine infants were thus enrolled, together with 10 who were referred either subsequent to the mother's HIV antibody test being positive, or because of symptoms suggesting HIV disease. These 49 infants comprised the index group.

Twenty four infants born to HIV seropositive fathers but whose mothers remained seronegative were included as controls. Mothers were tested after self identification of high risk activities, and belonged

to similar socioeconomic groups as mothers who tested positive.

PROCEDURE AT FOLLOW UP

The procedure at follow up was identical for children in both index and control groups. Those infants identified antenatally were seen and examined at birth, at age 6 weeks, and 3 months, and at intervals of three months until the age of 2. Thereafter review occurred every six months if the child remained well. Others were seen according to the protocol from the time of referral.

At each visit, a history was taken according to a standard questionnaire and the child examined for symptoms and signs of HIV disease. Developmental screening was performed using the Denver developmental screening test. Length, weight, and head circumference were documented. Blood was then taken for laboratory tests.

LABORATORY METHODS

At intervals of three months HIV antibody was measured in both index and control children using an antiglobulin enzyme immunoassay (Abbott Recombinant HIV 1 ETA) followed by a competitive enzyme immunoassay (Wellcozyme). For index children, HIV 1 antigen was measured by quantified enzyme immunoassay (Abbott HTLV 3 antigen EIA). Lymphocytes were cultured for HIV every six months in all index children; positive results were confirmed by antigen capture.

Other investigations on peripheral blood included measurement of haemoglobin, total white cell and differential count, T lymphocyte subsets, platelet count, and immunoglobulin concentrations as previously described.[4 5]

The significance of various comparisons were tested using the χ^2 test, or Student's t test as appropriate.

Results

MATERNAL DATA

Characteristics of mothers of index and control children are shown in table 1. All were white. Thirty seven index mothers were infected through intravenous drug use and six were heterosexual contacts. No differences were seen between the two groups of women in the use of drugs during pregnancy, although more index mothers resorted to drug use after the birth of the child. No woman had AIDS or AIDS related illnesses before the delivery of the child, although one developed *Pneumocystis carinii* pneumonia during a third pregnancy that was terminated at 18 weeks' gestation. The remaining 42 index mothers were clinically free of HIV related symptoms. There were two sets of twins and four sibling pairs among the index children.

Table 1 *Characteristics of 43 index and 24 control mothers*

	Index *No (%)*	*Control* *No (%)*
Mean age at delivery (years)	24·1	23·6
Single mother	15 (31)	5 (21)
First born child	21 (43)	11 (45)
Drug use during pregnancy	14 (29)	2 (8)
Drug use since pregnancy	20 (41)	2 (8)*
Infant in alternative care	12 (24)	1 (4)

*$p<0.05$ (χ^2 test).

INFECTION STATUS OF INDEX CHILDREN

Four index children showed clinical evidence of HIV disease (table 2), two of whom were referred because of symptoms. All had positive cultures for HIV, with HIV antigenaemia and persistence of HIV antibody beyond 18 months of age. These children were definitely infected.

Of the remaining cohort, 11 were under 18 months of age and because of presence of maternal antibody, were classified as indeterminate status.[7] None had positive results for HIV antigen or culture. Of 38 index children over the age of 18 months, 34 were HIV antibody negative, HIV antigen as well as culture negative. They were clinically free of symptoms that suggest HIV disease and have normal tests of immune function. These 34 children were presumed uninfected.

PERINATAL DATA

Table 3 summarises perinatal data of index and control children, with the index cohort separated into children who were infected and those presumed uninfected. The mean birth weight was significantly lower in index children presumed uninfected (95% confidence interval (CI) 2714 to 3078 g) when compared with controls, (95% CI 3069 to 3527 g) and the difference remained when all children whose mothers used drugs during pregnancy were excluded from analysis. The effect of preterm delivery was allowed for by extrapolating preterm birth weight to term, along the same centile, and index children presumed uninfected were significantly lighter than controls.

All but one index infant were delivered by spontaneous vaginal delivery. Neonatal special care was required mainly for drug withdrawal symptoms and intrauterine growth retardation. Intensive care was only necessary for two index infants because of problems arising from preterm delivery. These consisted of meningitis with septicaemia resulting in ventriculoperitoneal shunts for hydrocephalus

CLINICAL OUTCOME

The median age when last seen was 24 months for index infants (range 3 to 52 months), 12 months for controls (range 3 to 30 months). No child has been lost to follow up. Within the index group, children whose infection status was indeterminate have been excluded from analysis. Signs and symptoms observed in index and control children are shown in table 4, with index children separated into those infected and presumed uninfected.

Appreciable lymphadenopathy was defined as the presence of nodes >0·5 cm in diameter in more than two non-contiguous sites (excluding inguinal) and persisting beyond three months. Chronic or recurrent respiratory infections were those which occurred on three or more occasions within a three month period. Recurrent diarrhoea was defined as loose stools that persisted beyond 48 hours, required treatment, and occurred on more than one occasion per month.

Failure to thrive was diagnosed when the child's sequential weights declined across centile lines.

As seen in table 4, signs and symptoms were non-specific and were seen in control as well as index children. Lymphadenopathy and recurrent respiratory infections were seen in appreciably more index children, even those presumed uninfected, when compared with controls. All index children presumed uninfected had symptoms and signs which resolved with time; they never had positive HIV antigen or culture results.

Neurological signs were detected only in the child with neonatal meningitis (ataxic diplegia). The developmental progress of the remaining cohort was within normal limits.

LABORATORY RESULTS

Laboratory abnormalities are shown in table 5. No significant differences were seen when the index children were compared with controls. Within the index children, however, those presumed uninfected were significantly less likely to have persistent hypergammaglobulinaemia, lymphopenia, T4 lymphopenia, and thrombocytopenia. Abnormal laboratory results in index children presumed uninfected tended to be transient findings which returned to the normal range on repeat testing.

Sequential laboratory data within the index group are displayed in table 6. Hypergammaglobulinaemia was noted as early as 6 months, and often predated

Table 4 *Clinical signs and symptoms in 38 index and 24 control children*

	Index		*Control (n=24)*
	Infected (n=4)	*Presumed uninfected (n=34)*	
Significant lymphadenopathy	4*	9*	0*
Recurrent respiratory infections	4	13*	2*
Recurrent diarrhoea	4*	11*	5
Eczematous rash	3	7	1
Hepatosplenomegaly	4*	4*	0
Failure to thrive	1	3	0

*$p<0·05$ (χ^2 test). Other comparisons between index and control children, as well as within index children, did not reach significance.

Table 5 *Laboratory abnormalities in 38 index and 24 control infants*

	Index		*Control (n=24)*
	Infected (n=4)	*Presumed uninfected (n=34)*	
Hypogammaglobulinaemia	0	13	4
Hypergammaglobulinaemia	4*	4*	4
Neutropenia ($<1·0\times10^9$/l)	1	2	3
Lymphopenia ($<2·8\times10^9$/l)	3*	4*	2
T4 lymphopenia ($<1·0\times10^9$/l)	3*	0*	1
T4/T8<1·0	4*	5*	1
Thrombocytopenia ($<100\times10^9$/l)	4*	4*	0

*$p<0·05$ (χ^2 test).

transmission, as estimated from our data, was 7·1%, lower than that previously reported.[4–6] This could be explained, in part, by the fact that all the infected women were free of HIV related symptoms during pregnancy. Testing for HIV antigen was performed in eight mothers during pregnancy, and in 22 after delivery of the infant—all were found to be negative. Our results must be interpreted with caution, as the numbers are small and as yet, the prognosis for children aged over 18 months who lose maternal antibody is unknown. Although not observed in our cohort, children have been reported to lose antibody only to seroconvert later on in childhood,[14] while others have been documented to remain antibody negative with positive antigen tests and virus culture.[4 5 15]

Recent developments in diagnostic techniques, such as in vitro production of HIV specific antibody,[16] or polymerase chain reactions for amplification of viral DNA,[17] are still under evaluation and not routinely available. A recent study found that six of 14 asymptomatic newborns, and five of 10 seronegative children of infected mothers were positive for HIV-DNA, using the polymerase chain reaction technique.[18] Using more sophisticated tests, the numbers of infected children in our cohort could be higher. There is, however, an urgent need for improved methods to evaluate the seronegative index children who remain clinically well with normal immune function, as four mothers have embarked on subsequent pregnancies based purely on the observation that the index child was symptom free and antibody negative (presumed uninfected).

We were unable to detect any laboratory predictor of HIV disease when sequential index and control data were compared. In the children with proved infection, immunoglobulin concentrations (especially IgG) did, however, start to rise before clinical signs were obvious. Clinical manifestations are non-specific, but when seen in conjunction with laboratory abnormalities (hypergammaglobulinaemia, T4 lymphopenia, thrombocytopenia), a diagnosis of HIV infection can be strongly suspected in a child especially when mother has engaged in high risk activities.

In future, we hope to use the polymerase chain reaction to detect HIV-DNA in the index children presumed uninfected. It must be stressed that the demonstration of viral DNA sequences does not imply overt infection, as little is known about the immune response to HIV in children infected antenatally. Our study stresses the importance of continued careful follow up of all children at risk of HIV infection, to detect early markers of infection as well as to determine the outcome of those who appear symptom free.

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Intravenous immunoglobulin in HIV infection: evidence for the efficacy of treatment

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SUMMARY Eight children with symptoms of HIV infection were treated for 12–26 months (median 14 months) with infusions of intravenous immunoglobulin (200 mg/kg) every three weeks. Significant improvement was noted in all children in terms of weight gain, number of infectious episodes, and days spent in hospital. This resulted in a 49% saving in cost on treatment compared with costs accrued previously during inpatient admissions. Immunoglobulin concentrations, which were raised at the start of treatment were not altered, and T4 counts continued to decline slowly. HIV core antigen was detected in four children before treatment, but all became core antigen negative after treatment was commenced, this effect being sustained in three. Intravenous immunoglobulin therefore has major clinical benefit, and by reducing viral activity may delay disease progression.

HIV infection in infants and children is associated with high morbidity and mortality,[1] and present therapeutic options are limited to treatment for specific infections,[2] and to zidovudine, which has many documented side effects and unknown long term sequelae.[3] As in adults, these infants may present with opportunistic infections and features suggestive of deficiencies of cellular immunity, with reversed T4/T8 ratios.[4 5] In addition, however, children commonly present with recurrent bacterial infections,[1 6] and defective humoral immunity has been shown.[7] Although serum concentrations of IgG, IgA, and IgM are typically raised,[8] panhypogammaglobulinaemia has been described.[9–11] Even in the presence of hypergammaglobulinaemia, which is attributed to polyclonal B cell activation,[12] patients behave as though they were hypogammaglobulinaemic. Hence passive immunisation in the form of intravenous immunoglobulin has been reported to be of benefit.[13 14] The aim of this study, therefore, was to make a fuller evaluation of the clinical effect of at least 12 months' treatment, to document any changes in laboratory indices of viral activity, and to assess the financial implications of committing children to maintenance treatment with intravenous immunoglobulin.

Patients and methods

Eight patients with clinical and laboratory evidence of HIV infection were identified, five of whom have been described in a preliminary report.[15] Clinical features at the start of intravenous immunoglobulin treatment are summarised in table 1. Children were treated with intravenous immunoglobulin on the basis of a history of two or more episodes of bacterial pneumonia, a three month history of recurrent or chronic upper respiratory tract sepsis or diarrhoea, or both, or symptomatic thrombocytopenia, with laboratory confirmation of HIV infection. All were treated every three weeks with 200 mg/kg of intravenous immunoglobulin supplied by Scottish National Blood Transfusion Service on a hospital day patient basis.[16]

The procedure and laboratory methods adopted at each visit were as previously described.[17]

The financial implications were calculated on the basis that the cost of intravenous immunoglobulin for a 15 kg child is £45 and materials, medical, nursing, and administrative staff time is £40; this gives a total of £85 per visit, or £1600 per year. The cost of inpatient paediatric care in south east Scotland is £960 per week.[18]

Table 1 *Clinical data*

Patient No	*Mode of transmission*	*Age at start (months)*	*Time on intravenous immunoglobulin (months)*	*Stage**	*Clinical features*
1	Vertical	9	26	P2A	Recurrent upper respiratory sepsis, diarrhoea, eczema, failure to thrive
2	Vertical	24	23	P2A	Recurrent upper respiratory sepsis, recurrent pneumonia, diarrhoea
3	Vertical	27	17	P2B	Prolonged pertussis, encephalopathy, recurrent pneumonia, diarrhoea
4	Vertical	48	17	P2D3	Recurrent pneumonia, diarrhoea, oesophageal candida, failure to thrive
5	Blood transfusion	42	16	P2C	Purpura, pneumonia, oral candida, lymphocytic interstitial pneumonitis
6	Vertical	26	14	P2C	Upper respiratory sepsis, recurrent pneumonia, lymphocytic interstitial pneumonitis, eczema
7	Vertical	36	14	P2F	Purpura, night sweats, upper respiratory sepsis, pneumonia, eczema
8	Vertical	12	12	P2F	Purpura, recurrent fever, upper respiratory tract sepsis, eczema

*Stage according to the Centers for Disease Control criteria.[25]

Results

After 12 months on treatment subjective improvement in general well being was reported in all eight patients. Case 3 showed an initial dramatic improvement in his encephalopathy, as previously described,[19] but later deteriorated to become severely handicapped before finally succumbing to a pneumonic illness 17 months into treatment. The other seven children remain well enough to attend normal school (n=2) or nursery (n=5) regularly. Table 2 summaries clinical findings before and after treatment. The number of infections sufficiently severe to require hospital review was significantly reduced on treatment, as was recurrent diarrhoea. Improvement in weight gain was seen in the cohort as a whole ($p<0{\cdot}01$); the most dramatic change was seen in those children in whom failure to thrive had been a presenting feature.

The total number of days spent in hospital for all eight children in the 12 months before treatment (except for case 1 in whom only nine months from birth had elapsed) was 220 (range 0–60), compared with 56 (range 0–20) in the first 12 months on intravenous immunoglobulin ($p<0{\cdot}001$). Assuming an unchanged rate of infection, the difference between actual and expected number of days of hospitalisation would be 181·6; this would result in a saving on inpatient costs of £24 905 compared with treatment costs of £12 800. Thus the net saving in costs of £1513/patient/year, or 49%, even without taking into consideration the travelling costs and time lost to the family in attending hospital.

Symptomatic thrombocytopenia, seen in three patients before treatment showed no immediate improvement on this dose of intravenous immunoglobulin, and a further child developed purpura while on treatment. Hypergammaglobulinaemia,

Table 2 *Clinical outcome after treatment*

	Before (median)	*After (median)*	*p Value*
Range of weight velocity (g/month)	−212 to +312 (117)	150 to 582 (300)	<0·01*
Total No of serious infectious episodes	34 (3)	6 (1)	<0·05*
Total No of episodes of pneumonia	13 (2)	4 (0)	NS
Total months of recurrent or chronic diarrhoea	49 (9)	21 (3)	<0·05*
No of patients with:			
Upper respiratory tract sepsis	8	8	NS
Eczema	5	5	NS
Significant lymphadenopathy	8	6	NS
Hepatosplenomegaly	7	8	NS
Thrombocytopenic purpura	3	2	NS

*Comparison of symptoms and signs experienced by the cohort in the 12 months before and in the subsequent 12 months after treatment.

involved, further studies will be required to verify these findings. The mechanism is likely to be very different, as plasma donors are screened for HIV infection and the intravenous immunoglobulin preparation has no anti-HIV antibody. It has been suggested that the production of HIV antigen is an indicator of enhanced expression of viral genes,[23] and may reflect the expression of latent virus after activation of helper T cells. Bacterial and viral infection increase T cell activation, and the disappearance of p24 antigen after commencing treatment may be due to the reduction of viral activation after the decrease in infective episodes. The continued decline in the T4 count was disappointing, but the disappearance of core antigen with intravenous immunoglobulin treatment suggests that the progression to AIDS may be delayed, as the loss of anti-p24 antibody and the appearance of core antigen are recognised bad prognostic signs.[24]

The clinical benefit of treatment we have shown, together with the quality of life enjoyed by our patients, leads us to conclude (in agreement with Schaad's group[19]) that there is no case for withholding intravenous immunoglobulin treatment in symptomatic children. This precludes a double blind placebo controlled trial at this stage, although further studies may be necessary to optimise the dose regimen. What remains unclear, however, is at what point in the course of disease treatment should be commenced, given that five of our children were in the early stages of symptomatic disease (CDC P2 A/F).[25] A controlled trial will be necessary if we are to consider treatment in asymptomatic children. This is liable to be problematic, however, because at present there is still no reliable diagnostic test of HIV infection in young children. As the problem of HIV infection in children grows over the next few years, routine intravenous immunoglobulin treatment has major implications in budgeting of hospital inpatient and day care facilities. We have shown the cost benefit of treatment—at least in the short and medium term. In is unlikely, however, that intravenous immunoglobulin will delay progression indefinitely, and further advances in immunotherapy, such as the introduction of specific anti-HIV neutralising antibody in combination with intravenous immunoglobulin and anti-retroviral treatment, will be required if we are to improve the ultimate prognosis for HIV infected children.

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The polymerase chain reaction in the diagnosis of vertically transmitted HIV infection

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The presence of HIV-1 DNA sequences in DNA from peripheral blood mononuclear cells (PBMCs) was investigated in a two-stage polymerase chain reaction ('double' PCR) using four sets of nested primers. The PBMCs tested were obtained from 46 children born to HIV-seropositive mothers, seven 'control' children born to HIV-seronegative mothers and seropositive fathers, and 45 healthy adult blood donors who were HIV seronegative. Nine of the children had symptomatic HIV infection and other laboratory features characteristic of HIV infection: all nine were PCR-positive with each set of primers in each of their 22 blood samples tested. The remaining 44 children had no clinical or laboratory evidence of HIV infection, and each of their 50 samples was PCR-negative with each set of primers, as were all blood donor samples. PCR-positive samples were tested in more detail using two of the sets of primers, which spanned hypervariable regions in the *env* gene. Polyacrylamide gel electrophoresis of DNA amplified from these regions yielded patterns of amplified DNA length variation which were characteristic for each child, and which changed little with time (in serial samples obtained over periods of 3–7 months). This excluded contamination as a cause of PCR positivity. This is the first report of the use of a double PCR for the diagnosis of HIV infection. The results demonstrate the specificity of this PCR method in diagnosis, with failure to reveal in this cohort any cases of vertically transmitted HIV-1 infection in addition to those already confirmed by conventional laboratory techniques.

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Keywords: HIV, polymerase chain reaction, nested primers, hypervariable regions, diagnosis, vertical transmission.

ntroduction

The laboratory diagnosis of HIV infection in children is usually made by demonstration of the persistence of circulating HIV antibody beyond 18 months of age, by culture of HIV, or by the detection of HIV core (p24) antigen. Other abnormalities present, such as hyper- or hypogammaglobulinaemia or an abnormal CD4 (helper) lymphocyte count, may also facilitate diagnosis [1]. In the asymptomatic child, however, it may be difficult to confirm HIV infection as circulating transplacentally acquired maternal antibody may take up to 18 months to clear [2], and other laboratory tests may be negative for antibody [3,4], HIV p24 antigen or virus culture [5].

The polymerase chain reaction (PCR) is an *in vitro* method of selectively amplifying specific DNA sequences [6,7]. It has been used successfully to detect the presence of HIV proviral DNA in the peripheral circulation of HIV-infected individuals [8,9]. PCR may allow detection of HIV infection prior to seroconversion [10] and has been reported to detect latent seronegative infection in

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dults [11–13] and to enable diagnosis of HIV infection n children [14–16].

A prospective study of HIV infection in children born to HIV-seropositive mothers is currently in progress in Edinburgh [17]. In the course of this study, several diagnostic ndicators of HIV infection in infants are being compared. We report here the results of a comparison of PCR analysis of DNA extracted from peripheral blood with other nethods of laboratory diagnosis in children at risk of HIV nfection.

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atients

ifty-three children were studied. Nine had symptomatic IIV infection, according to the Centers for Disease Control (CDC) paediatric classification of HIV infection [1] group 1); 29 were asymptomatic children born to HIV-nfected mothers but from whose circulation maternal antibody had cleared and who tested negative for serum IIV p24 antigen and HIV culture (group 2); eight were hildren born to seropositive mothers but from whose irculation maternal antibody had not cleared (group 3), nd seven were children born to seronegative mothers vith seropositive fathers (group 4). The negative control amples studied were from 45 healthy HIV-1-seronega-ve blood donors with no risk factors for HIV infection group 5).

erology, HIV p24 antigen assay and virus culture

erum samples were tested for HIV-1 antibody by en-yme immunoassay (Wellcome Diagnostics, Dartford, JK, and/or Abbott Diagnostics, Maidenhead, UK) and IIV p24 antigen by antigen-capture assay (Abbott). Pe-pheral blood mononuclear cells (PBMCs) were sepa-ated over Ficoll-Hypaque and cultured in RPMI-1640 cul-ure medium (Gibco Europe Ltd, Uxbridge, UK) supple-nented with 10% fetal calf serum, 2% penicillin and strep-omycin, 80 U/ml recombinant human interleukin-2 (Du ont, Stevenage, UK), and 3 µg/ml phytohaemagglutinin. ulture supernatants were harvested at weekly intervals nd tested for the presence of p24 antigen (Du Pont). eactive samples were confirmed by neutralization [18].

lood samples

wo-millilitre blood samples were collected into thylenediamine tetraacetic acid (EDTA) and PBMCs ere separated over Ficoll-Hypaque. PBMCs were ashed once in normal saline and the cell pellet was ored at −20°C for up to 12 weeks. The DNA from the ell pellets was extracted in 0.4 ml lysis buffer (50 mmol/l DTA, 0.1 mol/l NaCl, 50 mmol/l Tris HCl) containing 1% arcosyl and 100 µg/ml proteinase K, followed by extrac-on with phenol (twice) and chloroform prior to precipi-tion in 70% ethanol overnight at −20°C. After resuspension in distilled water and quantification by 260 nm light absorption, 1 µg aliquots of DNA (equivalent to 150 000 cells) were used for each PCR. PBMCs from 5 ml samples of EDTA-anticoagulated donor blood were handled identically. The preparation of cell pellets, DNA extractions, and PCRs were performed on donor and paediatric blood samples concurrently, in order to be able to detect any cross-contamination during each experimental stage.

Polymerase chain reaction

A two-stage PCR (double PCR) was performed, using four pairs of HIV-specific primers [19]. In the first PCR, 1 µg target template DNA was present in 50 µl 67 mmol/l Tris-HCl (pH 8.8), 16.7 mmol/l ammonium sulphate, 6.7 mmol/l $MgCl_2$, 10 mmol/l 2-mercaptoethanol, 6.7 µmol/l EDTA, 33 µmol each of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP) and deoxythymidine triphosphate (dTTP), 170 µg/ml bovine serum albumin, 10% dimethylsulphoxide, 0.5 µmol/l each outer nested primer and 0.015 U/µl Taq polymerase (Cetus). The target DNA was amplified using a 'Cetus' (Perkin-Elmer Cetus, Beaconsfield, UK) thermal cycler; 25 heat cycles were used, each of 0.6 min at 94°C, 0.7 min at 50°C and 3 min at 68°C. One microlitre of the reaction mixture from the first PCR was then transferred to a second tube containing 20 µl of the same medium as before, but with a second pair of primers lying within the region amplified initially, and a further 25 heat cycles were carried out with the same programme. Recombinant HIV-1 plasmid positive (pBH10.R3) and herring sperm DNA negative controls were included in each PCR assay.

Four outer primer pairs complementary to different parts of the HIV genome were used simultaneously in the first PCR reaction, and the four inner primer pairs were subsequently used singly in four separate second PCR reactions. The four amplified sequences were in the *gag*, *pol*, and *env* regions of the genome. Two of the primer pairs spanned hypervariable sequences in the *env* region. Details of the method are reported elsewhere (P. Simmonds *et al.*, submitted for publication). The sequences used were: *gag* primers 881: 5′-GGTACATCAGGCCATATCACC sense 1214 base pair (bp); 882: 5′-ACCGGTCTACATAGTCTC antisense 1669 bp; 883: 5′-GAGGAAGCTGCAGAATGGG sense 1407 bp; 990: 5′-GGTCCTTGTCTTATGTCCAGAATGCTG antisense 1646 bp: *pol* primers 001: 5′CATGGGTACCAGCACACAAAGG sense 4149 bp; 004: 5′-TCTACTTGTCCATGCATGGCTTC antisense 4380 bp; 002: 5′GGAGGAAATGAACAAGTAGATAAATTAGTCAG sense 4175 bp; 003: 5′-TCACTAGCCATTGCTCTCCAATT antisense 4290 bp; *env1* primers 401: 5′GAGGATATAATCAGTTTATGG sense 6539 bp; 404: 5′-AATTCCATGTGTACATTGTACTG antisense 6976 bp; 402: 5′GATCAAAGCCTAAAGCCATG antisense 6560 bp; 403: 5′CAATAATGTATGGGAATTGG antisense 6876 bp; *env3* primers 406: 5′-TCAGGAGGGGACCCAGAAATT sense 7316 bp; 247: 5′-GATCCCATAGTGCTTCCTGCTGCT antisense 7816; 407: 5′-GGGGAATTTTTCTACTGTAAT sense 7361; 405: 5′-TATGAGGGACAATTGGAGAAG antisense 7665 bp.

The 20 µl product of the second PCR was electrophoresed on a 3% low-melting-point agarose gel containing ethidium bromide to detect amplified product DNA. With this detection method, the presence of HIV-1 DNA in the initial sample is indicated by the presence of a single band of the correct size following electrophoresis. This PCR method has a sensitivity sufficient to detect a single molecule of target HIV DNA in up to 5 µg human DNA [19]. In earlier experiments, PCR amplification (using human primers) of human DNA sequences prepared by this method gave consistently positive results [19], validating the method of DNA preparation.

To assess length polymorphisms in the *env* region in samples found HIV PCR positive by the above method, the second PCR amplifications with the *env1* and *env3* primers were repeated in the presence of 5 µCi ^{35}S-dATP (Amersham International plc, Aylesbury, UK) per 20 µl reaction volume. To increase the incorporation of labelled nucleotide in the amplified DNA, the concentration of unlabelled triphosphates was reduced to 8.25 µmol/l. The product was electrophoresed on denaturing polyacrylamide gels. The molecular weight of the amplified DNA was estimated using appropriate markers.

Results

Each DNA sample studied was amplified with four pairs of outer primers, and 1 µl aliquots of the first PCR product were then further amplified with each of the corresponding inner primer pairs in four separate second PCR amplifications. The products were analysed by agarose gel electrophoresis and the presence of DNA indicated by staining with ethidium bromide. Samples were scored as positive or negative by the presence of a DNA band of the appropriate molecular weight in the product of the second reaction. With the double PCR, negative samples do not produce visible bands. Because of the high yield of DNA from all positive samples, each reaction therefore yields a clear positive or negative result. Table 1 records the results for the five groups of cases studied in each of the regions amplified. All 22 of the samples taken from the children in group 1 gave positive results with each of the primer sets, while none of the group 2 (n = 32), group 3 (n = 11), group 4 (n = 7) or group 5 samples gave positive results with any of the primers. Figure 1 illustrates the results of samples tested.

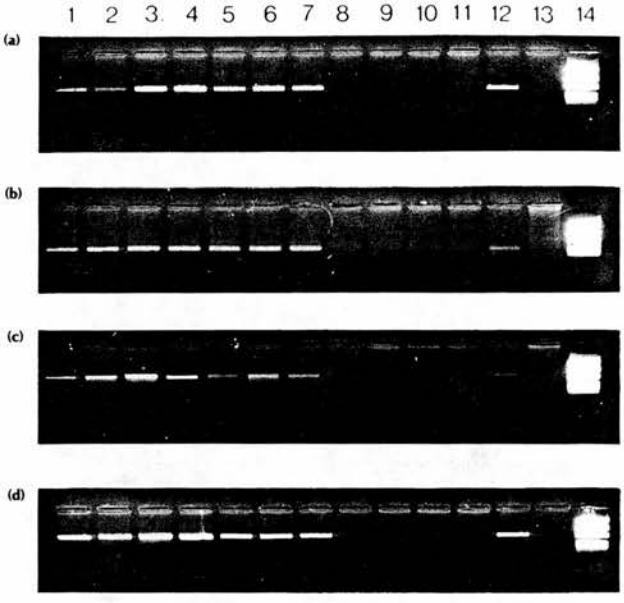

Fig. 1. Results of testing peripheral blood mononuclear cell (PBMC) DNA from children at risk for HIV infection with four sets of nested primers as follows: (a) *gag* primers; (b) *pol* primers; (c) *env1* primers; (d) *env3* primers. Columns 1–7: seven seropositive children (cases 9, 7, 8, 6, 5, 1, 4). Columns 8, 9: two seronegative children. Columns 10, 11: two serologically indeterminate children. Column 12: positive control (65 ag of plasmid pBH10.R3). Column 13: negative control (negative T- lymphocyte cell line 08166). Column 14: molecular weight markers (pTZ18R, *Hae*III digest; bands of 767, 458/434, 281/267, 174 and 142 base pairs are visible.

All PCR-positive samples identified in this study were amplified using primers which span the first and third hypervariable regions of the *env* gene [20]. Polyacrylamide gel electrophoresis of the ^{35}S-labelled PCR product showed a range of length variants to be present in each sample (Fig. 2). Each pattern of variants is specific for each patient, and distinct from that of the cloned HIV sequence shown. This excluded contamination by sample mixing or by recombinant DNA as a cause of PCR positivity.

To investigate the reproducibility of this method, four aliquots of a DNA sample from each of the PCR-positive

Table 1. Categorization of negative and positive polymerase chain reaction (PCR) results*.

Group	Age in months (mean)	Number of patients	Number of samples	Region of HIV-1 genome amplified (no. positive/no. tested)			
				gag	*pol*	*env1*	*env3*
1) Infected	2–66 (41.2)	9	22	22/22	22/22	22/22	22/22
2) Presumed uninfected	18–54 (34.1)	29	32	0/32	0/32	0/32	0/32
3) Indeterminate	1–13 (5.9)	8	11	0/11	0/11	0/11	0/11
4) Paediatric controls	10–36 (23.4)	7	7	0/7	0/7	0/7	0/7
5) Adult controls		45	45	0/45	0/45	0/28	0/28

*PCR amplification of the HIV-1 regions shown was performed on the number of samples shown. The *env1* and *env3* regions were amplified in only 28 of the 45 blood donors.

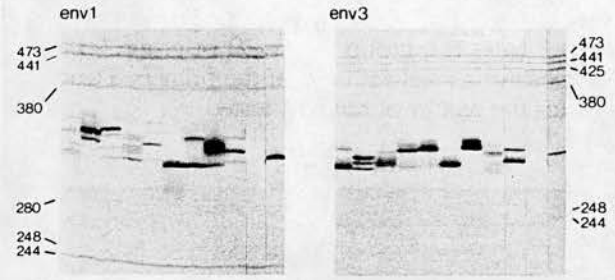

Fig. 2. Analysis of amplified DNA by high-resolution polyacrylamide gel electrophoresis. Radioactive polymerase chain reaction products in the *env1* and *env3* regions were electrophoresed to investigate length variation of the infecting virus strain within and between seropositives. From left, lanes 1–9: cases 1, 2, 3, 4, 5, 6, 7, 8, 9. Lane 10: seronegative child. Lane 11: 65 ag pBH10.R3 (single band). Size of markers in base pairs as indicated.

cases were amplified with the *env1* and *env3* primers in quadruplicate reactions. Representative results are shown in Fig. 3, where little variability in the pattern of amplified bands can be seen. Follow-up samples from each of the available infected individuals were similarly tested in order to investigate the stability of the observed patterns over time. Little variation in the pattern of bands was seen in samples collected over periods of 3–7 months, as illustrated by the samples from case 8 in Fig. 3.

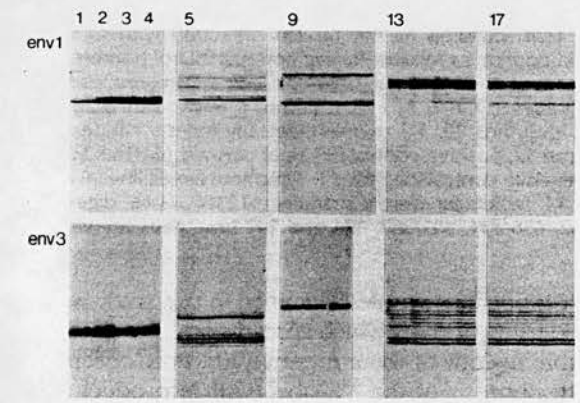

Fig. 3. Reproducibility of pattern of length variation in *env1* and *env3* regions. Lanes 1–4: four replicate reactions with DNA from case 6. Lanes 5–8: case 1. Lanes 9–12: case 7 (only three replicates of *env3* shown). Lanes 13–16: case 8. Lanes 17–20: case 8 venesected 3 months later.

Table 2 compares the PCR results with the results of serum antibody and HIV p24 antigen assays and virus culture, and also gives other relevant clinical and laboratory details of the cases studied. All nine children who were PCR-positive were HIV-1-antibody-positive by enzyme-linked immunosorbent assay (ELISA) testing (confirmed by Western blotting in each case). Four of the nine were HIV p24 antigen-negative at the time of PCR positivity (cases 1, 4, 5, 8), two of whom have never tested HIV p24 antigen-positive. The disappearance of HIV p24 antigenaemia in cases 1, 3 and 4 occurred in association with intravenous immunoglobulin G treatment [21]. Seven of the nine were HIV virus culture-positive on one or more occasion. Case 5 has remained persistently negative for both culture and serum HIV p24 antigen, despite being positive for antibody and PCR. The child has had hypergammaglobulinaemia and symptoms for 17 months, with a progressive decline in CD4 lymphocyte count. Case 6 was positive for serum p24 antigen aged 2 and 4 months, but umbilical cord blood cultured for HIV virus gave a negative result. The 29 group 2 and eight group 3 children, all of whom were PCR negative, have been asymptomatic with normal serum immunoglobulin levels and negative HIV p24 antigen assay at each 6-monthly follow-up clinic visit, and show no evidence of CD4 lymphocyte depletion where tested. These data constitute strong evidence for the absence of HIV infection in these cases.

Discussion

PCR offers considerable promise for the detection of vertically transmitted HIV infection, as the method might be capable of yielding diagnostic results with high specificity and sensitivity at an age when other methods of detection may not.

There have been three recent reports of the use of PCR in the diagnosis of vertically transmitted HIV infection, each using a single PCR amplification followed by identification of the (amplified) product with a radioactive oligonucleotide probe [14–16]. Laure *et al.* [14] amplified three conserved sequences in the *gag* (1) and *pol* (2) regions. Some samples were termed PCR-positive even when positive results were obtained with only one or two of the three combinations of primers used, and such results were seen even in children with proven HIV infection. HIV infection by PCR positivity was claimed in six of 14 newborn infants tested, but other laboratory confirmation of HIV infection was present in only one of these. Also, five out of 10 children aged 2–5 years were claimed to be HIV-infected on the basis of PCR positivity, although other laboratory confirmation in these cases was similarly lacking; moreover, all were HIV-antibody-negative. Rogers *et al.* [15] amplified two HIV sequences in the HIV p24 and HIV gp41 regions, but had a high false-negative rate with only six PCR-positive neonates found among 11 who later went on to develop AIDS. Edwards *et al.* [16] amplified two sequences in the *gag* region, but similarly had negative PCR results in one child with symptomatic HIV infection.

Other workers have also failed to find a clear correlation between indices of HIV infection and PCR reactivity. Imagawa *et al.* [13] found a number of individuals who were serum HIV-antibody and HIV p24 antigen-negative for several months, yet were PCR-positive on more than one occasion. Conversely, the finding of extremely low levels of provirus in certain individuals allows the possibility that false-negative results may occur by testing insufficient DNA. Simmonds *et al.* [19] report that PBMC DNA from one seropositive individual contained only five copies of provirus per 10^6 cells. Thus, considerably more than 1 μg DNA would need to be amplified to ensure re-

Table 2. Details of HIV-infected children.

	Cases								
	1	2	3	4	5	6	7	8	9
Age (months)	44	60	56	35	50	4 (D)	24 (D)	49	66 (D)
CDC stage	P2A	P2C	P2A	P2F	P2A	P2D1	P2B	P2C	P2D3
First symptoms	3	18	6	9	33	1	21	12	9
Zidovudine commenced	29	46	42	–	–	–	24	–	50–60*
HIV seropositive	0→	0, 18→	14→	0, 33→	0→	0→D	9→D	12→	9→D
Raised serum IgG	3+6+, 9−→	42+→	18+→	6+→	36+→	None	9+→D	26+→	44+→D
Raised serum IgA	3+→28−→44+	52+→	30+→41−→	6+15+→22−→	None	4+	15+→D	None	44+→54−→63+
p24 antigen +	6,9	42→	23, 53	3→12	None	2, 4	15→D	None	48→D
p24 antigen −	12→	None	27→52	15→	36→	None	9, 12	26→	None
HIV culture +	24, 26, 39	40, 47, 56	35, 36, 37, 42, 51	14, 17, 18	None	None	15	33	57
HIV culture −	40	None	None	None	39, 40, 45	Cord	None	None	None
PBMC PCR tested	39, 40, 41, 42	53, 56, 58	49, 53, 54	24, 27, 31	41, 43, 47	2	24	42, 45, 47	66

Age, age in months at end of study (July 1989) or at death (D); Centers for Disease Control (CDC) stage of HIV infection at the end of study or at death; first symptoms, age in months when symptoms started; zidovudine commenced, age in months zidovudine (AZT) started (in cases 1, 2, 3, 7, 9); HIV seropositive, age in months when serum HIV antibody-positive; raised serum IgG, age in months when polyclonal elevation in serum immunoglobulin G (IgG) concentration present (+) or not (−); raised serum IgA, age in months when polyclonal elevation in serum IgA concentration present (+) or not (−); p24 antigen +, age in months when p24 antigen detected in serum; p24 antigen −, age in months when p24 antigen not detected in serum; HIV culture +, age in months when virus grown from peripheral blood; HIV culture −, age in months when failure to culture virus from peripheral blood; PBMC PCR tested, age in months at which blood sample peripheral blood mononuclear cells (PBMC) taken for analysis by polymerase chain reaction (PCR) for HIV-1; →, same result as the last one shown obtained in each subsequent 3-monthly sample; →D, same result as the last one shown obtained in each subsequent 3-monthly sample, until death; None, these outcomes not shown in any sample tested; Cord, this outcome found in umbilical cord blood. *Zidovudine given from 50–60 months, then stopped because of leucopenia. All other children studied had normal total serum immunoglobulin concentrations, tested negative for p24 antigen and virus culture, and those aged over 18 months had become HIV seronegative.

producibly positive PCR results. It is likely that patients with yet lower amounts of PBMC provirus may be identified, and these may prove refractory to screening using PCR during such stages of infection. This emphasizes the importance of continued monitoring of all infants born to HIV-infected mothers. In all samples in this report scoring positive or negative, there were no discrepancies between the results obtained with the four independent sets of primers from *gag*, *pol*, *env1* and *env3*. The positive PCR results obtained were completely concordant with the clinical and laboratory evidence of HIV infection in these children. All control samples (groups 4 and 5) were PCR-negative, and no cases of latent HIV infection were detected in groups 2 and 3.

Mullis and Faloona [22] described the use of nested primers to increase the specificity of PCR for target sequencing. Simmonds *et al.* [19] have used this technique to improve the sensitivity as well as specificity of this method for HIV detection and have reported the reliable detection of single molecules of target sequence. This is considerably more sensitive than existing methodologies. Furthermore, the higher degree of amplification possible allows the direct visualization of amplified product, and also permits partial characterization of HIV by virtue of its strain-dependent length variation in several regions of the *env* gene.

The demonstration of different length variants of amplified HIV DNA is of considerable interest. The HIV genome exhibits great diversity in nucleotide sequence, both between isolates made from different patients and between multiple isolates made from the same patient [20,23–25]. In addition, certain segments of the region of *env*-encoding gp120 show extensive length variation [20,23]. In a separate study of HIV-infected haemophilic patients, Simmonds *et al.* have obtained nucleotide sequences which confirm the existence of the length variants present within patients' samples. The pattern of variants is similarly patient-specific (Simmonds *et al.*, in preparation). Thus it has proved possible to distinguish variants of HIV both between and within infected individuals, and to distinguish genuine positive reactions from contamination by exogenous HIV sequences.

The finding of different length-variant patterns in cases 7, 8 and 9 is of particular interest, as they are siblings. The different patterns seen imply either vertical transmission of different HIV *env* variants to each child, the rapid emergence of different variants within each child (possibly due to different immune selection pressures), or both. Unfortunately, library samples of the mother's PBMC DNA spanning the pregnancies are not available for analysis. PBMC DNA samples taken from haemophilic patients over intervals of 2 years can show considerable change in the patterns of length variants in the *env1* and *env3* regions in some individuals, while other individuals' patterns remain relatively unchanged (Simmonds *et al.*, in preparation). PCR positivity in case 4 is also of interest, as this patient is a dizygotic twin, the other twin being presumed uninfected (group 2) and PCR-negative.

Using a double PCR to amplify hypervariable regions of the HIV genome, we have been able to diagnose vertically transmitted infection with apparently 100% specificity and

100% sensitivity. The distinctive HIV DNA length-variant patterns observed might be usefully exploited in further studies as a basis for the investigation of the timing of vertical transmission, by comparing the patterns amplified from serial blood samples taken from the mother throughout pregnancy with those obtained from the infant at birth and during early infancy.

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Neurologic signs in young children with human immunodeficiency virus infection

HE EUROPEAN COLLABORATIVE STUDY*

Neurologic and neurodevelopmental problems ere investigated in a cohort of 39 human im-unodeficiency virus (HIV)-infected children nd 164 antibody-negative children born to IV-positive women. All children were fol-wed from birth for between 1 month and 4 ears. Serious neurologic manifestations were resent in 5 of 16 children (31%) who developed cquired immunodeficiency syndrome/acquired mmunodeficiency syndrome-related complex, though in 2 the neurologic signs were prob-ly not related to HIV. This can be compared ith a prevalence of 0 of 23 in children who mained asymptomatic or who had less severe IV-related symptoms or signs and 2 of 164 %) in uninfected children. Neurologic signs in e uninfected group were associated with the resence of drug withdrawal at birth and pre-aturity. These findings contrast with reports a high prevalence of neurologic findings in ost studies of HIV-infected children.

TRODUCTION

A wide range of neurologic manifestations related human immunodeficiency virus (HIV) infection ve been reported in children born to seropositive mothers. These include developmental delay, cognitive deficits, loss of developmental milestones, impaired brain growth, pyramidal signs, seizures, pseudobulbar palsy and more rarely ataxia, myoclonus and extrapyramidal rigidity. A high incidence (34 to 90%) of neurologic involvement has been described in children with acquired immunodeficiency syndrome-related complex (ARC)/acquired immunodeficiency syndrome (AIDS), and most have presented before 2 years.[1-3] HIV antigen and virus have been detected in cerebrospinal fluid and brain in children with perinatally acquired infection.[2,4] However, these studies are retrospective and biased towards symptomatic children; little is known about the prevalence of neurologic disease in HIV-infected children with less severe symptoms.

The aim of this study is to describe the prevalence and natural history of neurologic involvement and the temporal relation between neurologic manifestations and the progression of HIV disease in a cohort of children born to mothers with HIV infection.[5,6]

METHODS

The study was based on 350 children currently enrolled in the European Collaborative (EC) study and followed up to April 1989.[7,8] The children, all born to HIV-seropositive mothers in 8 European centers, were followed prospectively from birth. Infants were examined clinically in the first week of life and then every 3 months following a standard protocol including neurologic developmental as well as clinical and laboratory findings. All treatment was recorded, though the EC protocol contains no particular instructions on therapy. In each center a single pediatrician was responsible for clinical examinations. Most centers arranged detailed neurologic and developmental examinations, and in some these were conducted by specialists in these fields. What was recorded under the EC protocol were assessments of each child's language, personal/social, fine motor and gross motor development as "pass," "suspicious," "fail" or "loss of milestones." The neurologic assessments required were encephalopathy, paresis, abnormal gait, seizures and pathologic reflexes.

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Infection status was defined as the persistence of tibody after 18 months, the identification of virus antigen in antibody-positive children ages less than months or the presence of AIDS/ARC. Of the 350 ildren 39 were infected, 164 were antibody-negative d presumed not infected and in 147 the infection tus was indeterminate. This latter group has been cluded from the analysis.

The analysis relates the progression of HIV disease the onset of neurodevelopmental signs and symp-ms. The following definitions were used. AIDS was fined by the Centers for Disease Control, Atlanta, teria,[9] and ARC as in previous publications of the study.[5,6] In addition a definition was constructed significant HIV-related symptoms or signs, falling ort of AIDS/ARC, as two or more of the following: rsistent generalized lymphadenopathy, persistent patomegaly, persistent splenomegaly, chronic or re-rrent diarrhea, unexplained fever, failure to thrive, s of milestones or parotid swelling; or persistent l *Candida* infection. Unlike the definition of AIDS/RC, this definition of significant symptoms is not 0% specific. As a result symptoms in children who et this definition at any one time may resolve er.[10]

A child was defined as having serious neurologic ns if at least one of the following was reported on o successive examinations and had not resolved: cephalopathy (static/progressive); seizures; paresis; thologic reflexes; abnormal gait; loss of develop-ntal milestones. If any of these signs occurred on o examinations and then resolved, or if failure on least one component of the developmental assess-nt occurred (gross motor, fine motor, language, rsonal/social) on two successive examinations at st 2 months apart, this was regarded as evidence of inor neurologic or developmental problem.

Children were categorized according to the maternal tory of intravenous drug use: those with drug with-wal symptoms in the perinatal period; others whose thers had used drugs intravenously in pregnancy; l children of former intravenous drug users or thers with no history of intravenous drug use.

Time at risk of development of neurologic problems s the age when the criterial episode began, or age ast examination. Differences between groups in the idence rates of neurologic involvement are assessed exact one-tailed probabilities assuming a null hy-hesis of equal rates.[11]

SULTS

There were 203 children with known infection sta-in the study cohort. Thirty-nine were infected and who had lost antibody were presumed uninfected hey remained immunologically normal with no V-related signs or symptoms.

Their mothers were predominantly white (95%) and primiparous (65%), with a median age of 24 years (range, 16 to 38). Thirty percent lived alone and 84% were or had been injecting drug users. The median follow-up time was 18 months; 89% had been followed for more than 6 months, 67% more than 12 months and 28% more than 2 years. Over 90% of the children had been seen within 9 months of February 1, 1989, and this level of compliance was observed irrespective of infection status and mother's drug history.

Serious neurologic signs were present in 5 (13%) of the HIV-infected and in 2 (1.2%) of the 164 antibody-negative children. Minor signs were noted in a further 2 infected and 5 uninfected children.

Infected children. Sixteen of the 39 infected children developed AIDS or ARC, 14 had HIV-related symptoms or signs and 9 were asymptomatic. Five of the asymptomatic children had been followed for less than 1 year. Table 1 shows the relation between serious neurologic signs and clinical status when last seen. Neurologic signs were present in 5 of 16 children with AIDS or ARC, in 0 of 14 who had significant HIV-related symptoms or signs, and in 0 of 9 who were asymptomatic when last seen. The incidence of neurologic signs among children with AIDS/ARC was significantly higher than that in the other groups combined ($P = 0.004$).

Table 2 shows that four of the children who presented with serious neurologic signs already had AIDS or ARC before they met the definition of serious neurologic involvement. The fifth already had serious HIV-related symptoms. Three of these five children presented with a progressive encephalopathy: one with an intraventricular hemorrhage and one with persistent developmental delay. None of them had drug withdrawal symptoms.

Case 1 had no perinatal problems. He presented at 4 months with persistent oral candidiasis, lymphadenopathy and hepatomegaly (ARC) and mild motor delay. While hospitalized for cytomegalovirus interstitial pneumonitis at 5 months he had seizures and a further loss of developmental milestones. The cranial computer tomography scan showed bilateral low density areas with multiple round opacities in the basal ganglia region. There was no enhancement when using contrast medium. The child died of respiratory failure caused by cytomegalovirus pneumonia at 6 months.

TABLE 1. Serious neurologic signs in 39 HIV-infected children, by clinical status at last visit

HIV Disease Progression	*N*	Neurologic Signs	%	Total Time at Risk (Months)	Follow-up Time (Months)
Asymptomatic	9	0	0	149.4	3–47
HIV-related symptoms	14	0	0	285.0	1–39
AIDS/ARC	16	5	31	207.8	1–38

TABLE 2. Neurologic findings among 39 infected and 164 uninfected children

Case	Sex	Birth Weight (Kg)	Gestation (Weeks)	Findings	HIV Status at Onset[a]	Age Last Seen (Months)
ıted						
1	M	3.22	40	Encephalopathy Lymphoma (postmortem)	ARC	6[b]
2	F	1.84	37	Encephalopathy	AIDS	19[b]
3	F	2.65	41	Encephalopathy	AIDS	19
4	M	1.20	28	Intraventricular hemorrhage	Symptoms	12
5	F	2.34	36	Developmental delay	ARC	8[b]
ıfected						
6	F	2.15	36	Acute encephalopathy		22
7	M	1.68	31	Cerebral palsy		31

[a] HIV clinical status at onset of criterial neurologic episode.
[b] Died.

stmortem examination revealed interstitial pneumonia, fatty infiltration of the liver and disseminated omegalovirus infection. Frontal sections of the in showed a neoplastic deposit localized at each al ganglia; the histopathologic diagnosis was central nervous system lymphoma.

Case 2 was small for gestational age but was otherwise normal. ARC was diagnosed at 8 months followed persistent generalized lymphadenopathy, oral ndida infection and hepatosplenomegaly. At 10 nths AIDS was diagnosed as was also failure to ve, two episodes of sepsis (*Staphylococcus aureus* *Staphylococcus epidermidis*) and lymphocytic interstitial pneumonitis. By 16 months progressive encephalopathy was evident, with acquired microcely, and gross loss of developmental milestones. A nial computer tomography scan at 19 months wed severe cortical atrophy, attenuation of white tter and bilateral symmetrical calcifications of the al ganglia. The child died at 20 months with *Pneumocystis carinii pneumonia.* Postmortem examination he brain confirmed the cranial computer tomography scan findings. Microscopic examination showed or of the white matter, reactive astrocytosis, diffuse inflammatory cell infiltrates, perivascular calcifications in the region of basal ganglia and the presence of typical multinucleated cells, considered to be ted to HIV infection.

ase 3 had no perinatal problems and at 4 months eloped pneumonia of unknown origin that resolved r antibiotic therapy. At 5 months she presented n *P. carinii* pneumonia and at 10 months she had ertonia, pathologic reflexes and mild developtal delay. This developed to progressive encephathy with tetraparesis and severe developmental y at 14 months.

ase 4, a premature infant (28 weeks gestation, 1.2 developed Group B streptococcal sepsis at birth a bilateral intraventricular hemorrhage. At 4 ths he had a Group B streptococcal pneumonia at 6 months oral candidiasis, lymphadenopathy hepatosplenomegaly (ARC). AIDS was diagnosed at 10 months after a second episode of Group B streptococcal pneumonia. Head circumference and gross motor development were normal, though fine motor and language development at 12 months was considered impaired. He spent his first 4 months in the hospital before adoption. This infant's neurologic findings were not considered to be HIV-related.

Case 5 had no perinatal problems but at 6 weeks presented with *Klebsiella* sepsis (untyped), *Haemophilus influenzae* pneumonia, lymphadenopathy, oral candidiasis, hepatosplenomegaly, protein energy malnutrition and hepatitis B infection. At 3 months AIDS was diagnosed after a second bacterial pneumonia (*Streptococcus* Group B), and she died at 7 months. The child had been hospitalized since birth; fine motor, language and personal/social development had never progressed but gross motor development was normal.

There were a further two infected children in whom minor neurologic signs or developmental problems were reported. One with AIDS was noted to have abnormal reflexes during prolonged hospitalization while being treated with immunoglobulin and aztreonam, but these signs were no longer present after discharge. The other child with HIV-related symptoms had signs of general developmental delay for a 2 month period while institutionalized.

Among the 39 infected children 7 had been treated with zidovudine and immunoglobulin, and a further 7 had received immunoglobulin alone. Therapy was given to children with AIDS or occasionally to older children who had developed significant HIV-related symptoms for several months together with persistent immunologic dysfunction. Of those with serious neurologic involvement, all but one were treated after these symptoms started. Of those without serious neurologic involvement when last seen, only 9 (26%) were treated at all, and in 7 the therapy was begun in the second year of life, after the period when the onset of neurodevelopmental symptoms tends to occur.

Uninfected children. Case 6 had drug withdrawal symptoms at birth and at 3 months developed an acute

oatoencephalopathy of unknown origin.[12] This re-
ted in severe brain damage with epilepsy, cerebral
tonic palsy and mental retardation.
Case 7 was a premature infant with drug withdrawal
mptoms and neonatal meningitis. This resulted in
drocephalus requiring a ventriculoperitoneal shunt.
is infant had cerebral palsy with VI and VII cranial
ve involvement, spastic diplegia and ataxia. Devel-
mental progress was slow but satisfactory when last
n.
A further five uninfected children were reported to
e had signs of developmental problems which re-
ved. All five had drug withdrawal symptoms at
h. One child was noted to have pathologic reflexes
he 7- and 10-month examinations and gross motor
ay at 7 months. In the other 4 children psychomotor
/or language delay had been reported in a 2- to 4-
nth period. In one child these difficulties could be
ibuted to prolonged hospitalization for pneumonia
to her mother's suicide. A second child taking
oids for nephrotic syndrome and brought up by an
erate grandmother was reported to have a global
of milestones at 22 months. For the other children
re was no explanation for their developmental de-

The occurrence of neurologic or developmental
blems among uninfected children was associated
the presence of drug withdrawal. Among 41 un-
cted children with drug withdrawal, 6 had serious
inor neurologic signs or developmental problems
ing a total of 853 months at risk compared with 1
123 other children followed for a total of 2563
nths ($P = 0.0013$).
mong the 41 uninfected children with drug with-
wal, the 6 with serious or minor neurologic signs
e, on average, 0.55 kg lighter at birth (95% confi-
ce interval, 0.19 to 0.91), and were born on average
weeks earlier (95% confidence interval 1.8 to 5.3).
wever, the two groups did not differ in weight for
ational age.

CUSSION

this study 5 of 39 HIV infected children had
ous neurologic signs. This included one premature
nt with intraventricular hemorrhage, unlikely to
HIV-related, and another whose developmental
y is likely to have been a result of prolonged
pitalization. Depending on whether these latter
cases are regarded as HIV-related, the prevalence
erious neurologic signs was 8 to 13% in infected
dren, or 19 to 31% among children with AIDS or
C. All 5 children developed HIV-related disease, in
t cases severe, before any neurologic involvement
apparent. Although there have been reports of
-infected children whose initial presentation was
rologic,[13,14] the finding that neurologic manifesta-
tions appear after the diagnosis of AIDS/ARC has been reached is consistent with the majority of documented cases.[15]

The low proportion of children treated in this study and the timing of treatment relative to the onset of neurologic signs and symptoms make it very unlikely that treatment could have substantially altered the rate of development of neurologic illness.

The 164 children who lost antibody were presumed to be uninfected, although antibody loss has been reported in healthy but infected children.[6] Two of the children had serious neurologic signs, although not of a type previously associated with HIV, and both had experienced drug withdrawal symptoms in the perinatal period. Drug withdrawal was also associated with a higher risk of minor neurodevelopmental problems. This association may be a result of the direct effects of drug addiction or to continuing drug abuse which is likely to result in a less stimulating, more deprived environment for these children. The case histories at the time of onset tended to support the latter view.

Among infants with drug withdrawal in the perinatal period, neurologic signs were more likely to develop among those born earlier and smaller. This may reflect an increased vulnerability to the effects of prematurity in this group, or again prematurity could itself be a further marker of extreme social deprivation. Allowance must be made for preterm delivery in developmental assessment. There are conflicting views about the etiology of developmental problems in infants born to drug-dependent mothers[16–18] and it may not be possible to distinguish between the effects of drug use in pregnancy and social and obstetric factors.[19] Ultmann et al.[20] also stress that neurodevelopmental assessments of HIV-infected children must take into account both low birth weight and prematurity associated with maternal drug use, and the medical and social problems associated with drug use and HIV infection.

We report a lower incidence of neurologic involvement than in previous studies from the United States. In a study of 68 children from New York (median age, 16 months) with symptomatic HIV infection (50 AIDS, 18 ARC), 61 (90%) had central nervous system dysfunction.[1] These findings are similar to those reported in an earlier paper in a similar cohort in which 77% of the children with AIDS or ARC showed neurologic involvement.[2] What makes this discrepancy all the more striking is that in the European Study 3 of 5 children with severe neurologic problems died 2 to 7 months after the onset of AIDS/ARC and might not have been included in studies where follow-up began not at birth but at referral with symptoms. In the New York study it is possible that referral may have depended partly on the stage of HIV disease progression or even on the presence of neurologic signs.

In a report of 38 symptomatic HIV-infected children n France,[3] 8 children were described as having severe eurologic problems including mental retardation, notor delay and buccofacial dyspraxia, and a further had moderate neurologic involvement. The preva-ence of 21% severe neurologic abnormalities in chil-ren with AIDS or ARC is consistent with the present ndings. A subsequent follow-up of a larger number f children in this French study confirmed the close elation between the degree of neurologic involvement nd the level of immunodeficiency.[21]

In a prospective study Belmann et al.[22] compared ildren born to HIV-positive women in New York, ith others born to HIV-negative drug-abusing con-ols. Four of 9 of those remaining seropositive at 1 ear had neurologic or neurodevelopmental problems the first 24 months (maximum follow-up) compared ith 2 of 18 in the HIV-negative group. Although both roups have a higher prevalence than in the European udy, the sample sizes are too small to make a valid mparison. Nevertheless studies undertaken in the nited States consistently report a higher prevalence neurologic involvement in pediatric HIV patients an in European studies. It is not yet clear whether is is a result of methodologic differences in patient ecruitment, to different viral strains, to other cofac-rs or to the level of social and health care available. Further follow-up of children in the European Col-borative Study will show whether the pattern of eurologic involvement changes with increasing age nd will provide the opportunity to assess the more ubtle neurologic impairments in the infected and ninfected children.

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Diagnosis of Pneumocystis carinii pneumonia from non-invasive sampling of respiratory secretions

R A Hague, S E Burns, J Y Q Mok, P L Yap

Abstract

An infant infected with HIV presented with fever, tachypnoea, hypoxia, and radiological evidence of bilateral pneumonitis. Fluorescent antibody technique identified *Pneumocystis carinii* within 24 hours from secretions obtained by nasopharyngeal aspiration. This rapid, non-invasive method should be the first line investigation of suspected *P carinii* pneumonia in immunocompromised patients.

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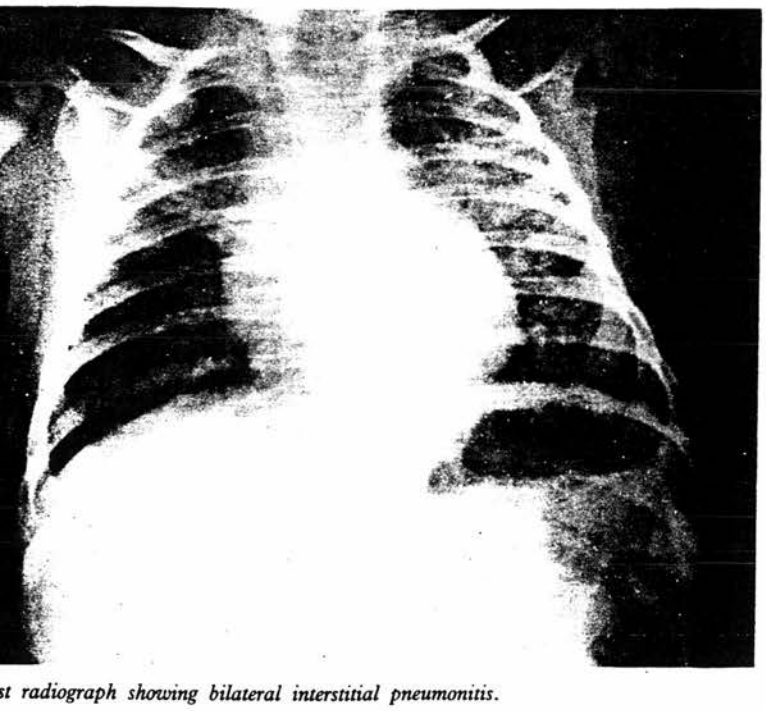
Pneumocystis carinii is an important pathogen in the immunocompromised child, and must be considered in the differential diagnosis of any such child presenting with respiratory symptoms. Characteristic radiological appearances are of diffuse reticulogranular infiltrates, particularly in the perihilar region, progressing to consolidation, but a normal chest radiograph is compatible with the diagnosis.[1] Cytomegalovirus or mycobacteria infection may produce similar findings.

Definitive diagnosis has previously relied on the demonstration of silver staining cysts in material obtained from open lung biopsy or bronchioalveolar lavage, requiring an invasive procedure in an acutely ill child.[2 3] Sputum induction in infants and small children is not a practicable technique,[4] and serology is unhelpful.[5] We report a case in whom the diagnosis was made from nasopharyngeal secretions using a fluorescent antibody technique.



st radiograph showing bilateral interstitial pneumonitis.

Case report

A white infant girl whose HIV seropositive mother developed *P carinii* pneumonia at 35 weeks' gestation was delivered at term weighing 3050 g. There were no neonatal problems, and her initial progress was complicated only by persistent candidal infection of the oral cavity and napkin area.

At 4·5 months she presented with a two week history of mild upper respiratory symptoms, two days of fever, an unproductive cough, and increasing tachypnoea, sufficiently severe to impair feeding. Examination showed a pale, mildly cyanosed and lethargic, but responsive infant. She had a tachycardia of 160/minute and respiratory rate of 80/minute, with bilateral intercostal and subcostal indrawing and scattered crepitations on auscultation. Lymphadenopathy and hepatosplenomegaly were noted and there was candidal dermatitis of the napkin area.

A chest radiograph showed extensive bilateral pneumonitis (figure). She was hypoxic (oxygen saturation 60%), with a carbon dioxide tension (PCO_2) of 5·37 kPa and hydrogen ion concentration of 39·7 mmol/l. On admission the neutrophil count was 6200×10^6/l, T4 lymphocyte count 1079×10^6/l, and T4/T8 ratio 1·4. Serum IgG concentration was normal (4 g/l), and IgA and IgM raised (0·9 g/l and 2·1 g/l). HIV p24 antigen was positive (140 pg/ml). Secretions obtained by nasopharyngeal aspiration were sent for microbiological investigation.

Pending results the infant received oxygen supplementation and high dose cotrimoxazole and acyclovir intravenously. The identification of *P carinii* was confirmed the next day (see below). The infant's condition initially stabilised, but deteriorated 24 hours later. Despite giving hydrocortisone and intravenous immunoglobulin, she failed to maintain oxygen saturation of >80% even with fractional inspiratory oxygen of 100%, and developed carbon dioxide retention (PCO_2 9·75 kPa) and respiratory acidosis (hydrogen ion concentration 73·7 mmol/l). Definite knowledge of the diagnosis, and its implications for the child's long term prognosis meant that the parents could be counselled appropriately. Their wish for her not to receive mechanical ventilation was respected, and she died on the fourth day of her admission. Postmortem examination confirmed the widespread alveolar exudate within the lungs in which round organisms were detected

by silver staining. No other organisms were detected.

Method

After addition of an equal volume of sputolysin (Behring Diagnositics) and glass beads the sample was vortexed for one minute and the incubated at 37°C for three minutes. A total of 10 ml of phosphate buffered saline (PBS) pH 7·2 was added and the mixture centrifuged at 2500 *g* for 20 minutes. The pellet was resuspended in 1 ml PBS and cytospin preparations made on two polyl-lysine coated slides. A cytospin preparation of positive rat lavage material was used as a positive control. After fixation for 30 minutes in equal volumes (50%) methanol and acetone, trypsin (0·25%) digestion for 10 minutes at 37°C followed. A standard wash procedure of three five minutes with PBS was carried out.

Mouse monoclonal antibody to *P carinii* (Northumbria Biologicals Limited) was added to the test and positive control slides and PBS only, and incubated for 40 minutes. After repeat washing antimouse IgG (F'ab) conjugated to fluorescein isothiocyanate was added and a further incubation of 40 minutes followed. After a last wash procedure substituting PBS pH 8·4 for the final change, the slides were mounted and viewed under ultraviolet light.

The sample was considered positive when three typical cysts showing all over fluorescence were seen, further confirmed by methenane silver treatment of the control slide.

Discussion

In HIV infection, the diagnosis of *P carinii* pneumonia has important implications, not only for the treatment of the acute illness, but also for longer term prognosis. In New York 13 out of 15 children less than 6 months old with *P carinii* pneumonia died during the acute episode, and all were dead by one year (J Chow, K Shah, K Li, *et al*; abstract presented at V International Conference on AIDS, Montreal, June 1989. Because of the risks involved in, and the technical expertise required for, paediatric bronchoscopy treatment is usually empirical. If the child dies, postmortem examination may give the answers, but if the child responds to high dose cotrimoxazole, the differential diagnosis between a bacterial pneumonia and *P carinii* pneumonia remains unresolved. This then creates difficulties in classification and staging of the child's disease, and in making therapeutic decisions, such as whether to commence zidovudine or prophylaxis for *P carinii* pneumonia.

Identification of *P carinii* pneumonia from upper respiratory tract secretions may only be possible in cases of overwhelming infection, and failure to detect it by this method does not exclude the diagnosis. It is in the most severe cases, however, that the patient is least able to tolerate more invasive procedures. Because our technique is rapid, other methods can still be used thereafter without undue delay. We believe therefore that it should be used as the first line investigation of suspected *P carinii* pneumonia.

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Cystic fibrosis, Pseudomonas aeruginosa, and selective decontamination

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n Dis Child 1990;65:1365–7).

Abstract

We used an oral topical antibiotic preparation to try and prevent oropharyngeal carriage of *Pseudomonas aeruginosa* in patients with cystic fibrosis. Ten of 15 patients treated with a two week course of intravenous ceftazidime together with a 90 day course of an antibiotic containing gel continued to carry *P aeruginosa* in the oropharynx.

The technique of selective decontamination of the digestive system uses oral antibiotic combinations to prevent the overgrowth of certain groups of organism (typically Gram negative aerobes), and allows the more usual flora to maintain colonisation resistance.[1] It has application particularly in the immunocompromised patient undergoing cytotoxic chemotherapy and in long term ventilated patients,[2] where Gram

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Case report

Hyperviscosity in HIV infected children – a potential hazard during intravenous immunoglobulin therapy

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Summary. A four year old boy with symptoms of HIV infection and serum IgG of 53.2 g/l had been treated for 4 months with regular infusions of intravenous immunoglobulin (IV IgG). During one such infusion he developed temporary neurological symptoms and signs suggestive of the hyperviscosity syndrome. Serum relative viscosity was raised at 5.0 (normal range 0.42–2.78). Subsequent IV IgG infusions given at a slower rate have been without adverse reactions. In a study of eight HIV infected children including the index case, and 20 children not infected with HIV, serum relative viscosity was significantly raised in the HIV infected children ($p < 0.01$; students t-test). Viscosity correlated with total serum IgG, which was raised in all HIV infected children, and with serum IgM. In HIV infected children with very high levels of serum IgG a slow rate of IV IgG infusion should therefore be chosen due to the possibility of hyperviscosity.

Key words: HIV infected children – Hyperviscosity – IgG

Introduction

Intravenous immunoglobulin (IV IgG) is now used in the treatment of Human Immunodeficiency Virus (HIV) infected children with recurrent bacterial sepsis, suggestive of defective humoral immunity [3, 5, 9, 10]. Although such children have a pattern of infections resembling those experienced by patients suffering from primary hypogammaglobulinaemia, serum levels of IgG, IgA and IgM are typically raised [1], and can be 3 or 4 times the upper limit of the normal range. It has previously been reported that a polyclonal hypergammaglobulinaemia may be associated with a raised serum viscosity in very rare cases [reviewed in 7], and we report on a HIV infected child in whom infusion of IV IgG lead to serious symptoms suggestive of hyperviscosity.

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Case report

The patient (Case 1, Table 1) became infected with HIV after blood transfusions at the age of 4 months, before screening of blood for HIV antibody was available. He remained well until 14 months when he began to develop recurrent upper respiratory tract infections, and at 21 months of age developed an immune thrombocytopenic purpura (ITP) refractory to treatment with high dose IV IgG, and to prednisolone. At the age of 3 years 6 months, his platelet count had returned to normal but he continued to have recurrent respiratory infections, failed to thrive, and had generalized lymphadenopathy and hepatosplenomegaly. The serum IgG, IgA and IgM levels were respectively 34.8, 2 and 2.8 g/l (normal ranges were 5–13, 0.47–2.63 and 0.36–1.92 g/l respectively). HIV infection was then diagnosed, based on HIV antibody detection and he was commenced on regular infusions of IV IgG manufactured by the Scottish National Blood Transfusion Service [5, 6] at a dosage of 200 mg/kg every 3 weeks, infused at 2.4 ml/kg/h at a IgG concentration of 50 g/l. This IV IgG preparation consists almost entirely of IgG with a normal distribution of IgG subclasses.

Treatment with IV IgG was not associated with any adverse reactions until the age of 4 yrs 10 months, when, due to an infusion pump malfunction, the patient received the infusion 30% faster than usual. He suddenly became confused, distressed, and was unable to see. He was dysarthric before becoming totally aphasic, and a transient right facial palsy was noted. Pupils remained equal and reactive to light, eye movements remained full and no abnormality could be detected on ophthalmoscopy. Tone, power and reflexes remained equal and symmetrical. The IV IgG was immediately discontinued and a normal saline infusion set up. After 30 minutes, he fell asleep. Two and a half hours later there was no residual neurological symptoms or signs. In retrospect, his parents commented that he had very brief episodes of confusion and drowsiness during previous IV IgG infusions, on one occasion waking up after a sleep appearing unable to speak. The effect had lasted a matter of minutes.

On the day of his severe adverse reaction his serum IgG, IgA and IgM levels were 53.2 g/l, 1.3 g/l and 4.8 g/l respectively. No paraprotein bands were found on protein electropheresis. His serum relative viscosity measured by capillary viscometer was 5.0 before and after the infusion (normal range 0.42–2.78). A computerized tomography scan subsequently showed no abnormality, and the patient has had no recurrence of symptoms suggestive of hyperviscos-

e 1. Serum viscosity and immunoglobulin levels of HIV infect-hildren

ber	CDC stage	Serum levels g/l IgG	IgA	IgM	Serum viscosity
	P2C	53.2	1.3	4.8	5.0
	P2F	29.6	3.3	2.4	3.5
	P2C	46.8	0.3	2.3	3.5
	P2A	14.8	1.6	1.1	3.0
	P2A	17.2	1.2	0.9	3.2
	P2A	18.8	0.7	1.2	3.6
	P2A	24.0	0.7	2.2	2.0
	P2D3	14.4	2.2	1.3	2.0

ontinuing his 3 weekly infusions at a slower rate of 1.3 ml/kg n. Subsequent viscosity measurements have varied between 3.0 5.0 (data not shown) and there was no difference in viscosity s before and after IV IgG therapy. Immune complexes were measured before and after IV IgG therapy and no change was rved.

Following this incident, we studied a further 7 children (Cases , Table 1) treated with IV IgG [8], who are described elsewhere of whom none had suffered side-effects associated with IV IgG sions. We also investigated 20 other children born to HIV sero-tive mothers who were being followed in the Edinburgh perina-ransmission study [6]. These children were presumed uninfect-being over 18 months old, HIV antibody negative, and free symptoms suggestive of HIV infection. Serum immunoglobu-evels were measured by laser nephelometry [6]. The mean D) viscosity in the HIV infected group was significantly raised a value of 3.2 ± 0.96 compared with the non-HIV infected p (2.3 ± 0.39; $p < 0.01$, student's t-test).

The relationship between serum IgG, IgA and IgM levels and serum relative viscosity was investigated in the above samples additional samples collected from the HIV infected children the serum relative viscosity was found to correlate both with levels ($r = 0.74$, $p < 0.001$) and with IgM levels ($r = 0.66$, 0.001). There was no significant correlation between serum vis-y and IgA levels (Table 1). In other HIV infected children, m viscosity was measured on stored samples taken before and ediately following IV IgG infusions. No rise in viscosity could emonstrated in any sample. Protein electropheresis revealed no oclonal or monoclonal bands in any of the specimens tested.

cussion

avenous immunoglobulin has been used widely for treatment of primary hypogammaglobulinaemia and . Adverse reactions are rare and in most clinical situa-s serum immunoglobulin levels are low or normal in recipients. In HIV infection, serum IgG levels prior to IgG infusion are usually high and problems with erviscosity have only once been previously described n HIV infected adult [7].

The hyperviscosity syndrome has been described in lts with paraproteinaemias [4] and in patients with oimmune and rheumatic diseases, when it is attribut-o aggregates of intermediate size or to polyclonal IgG ymers [11]. Although we have shown that the serum osity is raised along with serum immunoglobulin ls in these children, there have been no previous re-ts of hyperviscosity symptoms occuring after IV IgG sion. Clinical manifestations are rare in patients with viscosities less than 4 [12]. However, the 'symptomatic threshold' may be very variable, although remaining constant for any given patient. In this patient, the threshold was exceeded during one of the IV IgG infusions, and only the index patient developed symptoms during a rapid infusion of IV IgG.

Central nervous system (CNS) involvement by HIV as well as other infections (bacterial, viral or parasitic) have been documented [2]. We could not definitely exclude CNS infection in our patient as we did not have the opportunity to examine his cerebrospinal fluid. Nonetheless, it is possible that in a HIV infected patient with very high IgG levels, hyperviscosity might contribute to symptoms previously attributed to CNS infections and that methods of treatment which lower viscosity may be beneficial.

On the basis of our findings, we therefore recommend that in HIV infected children with very high serum immunoglobulin levels, the clinician is alerted to the problem of hyperviscosity. Caution should be exercised in these circumstances, and a slow rate of infusion of IV IgG chosen.

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THE LANCET

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ORIGINAL ARTICLES

Children born to women with HIV-1 infection: natural history and risk of transmission

EUROPEAN COLLABORATIVE STUDY*

600 children born to HIV-infected mothers by June 15, 1990, in ten European centres were followed to study the natural history of HIV infection and the vertical transmission rate. They were seen at birth, every 3 months up to 18 months of age, and every 6 months thereafter. At last follow-up, 64 children were judged to be HIV infected and 343 had lost antibody and were presumed uninfected. The initial clinical feature in infected children was usually a combination of persistent lymphadenopathy, splenomegaly, and hepatomegaly, though 30% of children presented with AIDS, or with oral candidosis followed rapidly by AIDS. An estimated 83% of infected children show laboratory or clinical features of HIV infection by 6 months of age. By 12 months, 26% have AIDS and 17% die of HIV-related disease. Subsequently, the disease progresses more slowly and most children remain stable or even improve during the second year. The vertical transmission rate, based on results in 372 children born at least 18 months before the analysis, was 12·9% (95% CI 9·5–16·3%). Virus has been repeatedly isolated in an additional small proportion of children (2·5%, 95% CI 0·7–6·3%) who lost maternal antibody and have remained clinically and immunologically normal. Without a definitive virological diagnosis, the monitoring of immunoglobulins, CD4/CD8 ratio, and clinical signs could identify HIV infection in 48% of infected children by 6 months, with a specificity of more than 99%.

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Introduction

Knowledge of the natural history of vertically acquired human immunodeficiency virus-1 (HIV-1) is based mainly on studies of children presenting with symptoms to specialist centres.[1-6] Prospective studies of vertically infected children should provide a more accurate understanding,[7-9] though increasing use of treatment could bias results. Information about earlier or less severe features of the disease and its progression is needed for the design of clinical trails, and to assist in management and early diagnosis of infected children. The European Collaborative Study (ECS) is a prospective study of children born to HIV-infected mothers.[10-12] We here report results relating to the natural history of HIV infection and the vertical transmission rate.

Children and methods

By June 15, 1990, 600 children born to HIV-1 seropositive mothers had been recruited and followed from birth in ten European centres (Padua, Berlin, Edinburgh, Madrid, Valencia, Amsterdam, Stockholm, Genoa, Brussels, Barcelona). Children were included only if their mothers were known to be infected at or before delivery; all such children were included.[10-12]

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Clinical, serological, and immunological examinations

At birth, gestational age, birthweight, and head circumference were recorded, in addition to information about maternal health, risk behaviours, and mode of delivery. Clinical and laboratory examinations were done. According to the protocol, clinical and developmental examinations were done and an interval history was taken every 3 months up to age 18 months, and every 6 months thereafter. Any examinations between scheduled clinic visits were also recorded. At each visit a blood sample was taken for the determination of HIV-specific antibody by enzyme-linked immunosorbent assay (ELISA) and western blot (WB); p24 antigen; total IgG, IgA, and IgM; and CD4 (T4) and CD8 (T8) lymphocyte subsets. Every 6 months, virus isolation was attempted in six centres. Repeat tests were done on separate samples when virus was isolated from antibody-negative children.

British and North American reference data were used to derive standard deviation (SD) scores for birthweight and head circumference at birth (controlling for gestational age and sex) and for weight and head circumference (controlling for age and sex). Approximate upper limits for IgG, IgA, and IgM were derived from the reference ranges used in the collaborating centres. An approximate lower reference standard for T4/T8 ratio (see footnote, table II) was derived from data on children who subsequently lost antibody, and in whom no virological evidence of infection was found.

The criteria for HIV infection were: acquired immunodeficiency syndrome (AIDS),[13] death considered to have resulted from HIV infection, persistence of antibody beyond 18 months, positive virus culture, or antigen detection in two samples. Lymphoid interstitial pneumonitis (LIP), as judged by severe respiratory symptoms confirmed by radiograph, was regarded as an AIDS indicator disease, but radiographic findings alone were not. The estimated vertical transmission rate was based on the 372 children of known infection status born at least 18 months before June 15, 1990.

Statistics

Multiple regression was used to estimate simultaneously the independent effects of HIV infection status, collaborating centre, and mothers' recreational drug taking on birthweight, head circumference, length of gestation, and SD scores. The categories used for infection status were: infected, presumed uninfected, and indeterminate; and for mothers' intravenous drug taking: baby with drug withdrawal, drug use in the final 6 months of pregnancy, previous drug user, and never used drugs. Statistical significance was assessed by χ^2 tests.

We estimated the incidence rates of signs and symptoms in infected pre-AIDS and uninfected children by dividing the number with at least one occurrence by the total time at risk. The definitions given in table II for each sign and symptom were used. Time at risk was age at first occurrence, age at the last visit, or age at AIDS onset, whichever was earliest. Time at risk of getting hyper-IgG, hyper-IgA, or hyper-IgM (hyper-IgG, A, M) was stopped at the onset of immunoglobulin therapy. The incidence rate ratio was the incidence rate in infected pre-AIDS children divided by the incidence rate in the uninfected children, and reflects the ability of the sign/symptom to discriminate between the two groups. The null hypothesis that the rate was not higher in infected children was tested with the one-tailed 'mid-P' probability test. The same methods were used in table III, except that risk time stopped at 12 months. The incidence rates in infected and uninfected children led to the following definition for HIV-related initial signs and symptoms (HIV-ISS): oral candidosis, or any two concurrently of lymphadenopathy, hepatomegaly, and splenomegaly, persisting for more than 2 months. Children whose initial presentation was AIDS were recorded as having HIV-ISS at that date.

Survival curves were calculated by non-parametric methods designed for interval-censored data[14] because changes in clinical or immunological status were not observed directly; they were seen only at the age when they were first detected and the age at the previous assessment. This method estimates for each age interval a the probability $f_{M,a}$ that the marker M first occurs at age a. The probability that no episode has occurred by age a, $S_{M,a}$, is $1 - \Sigma_{i<a} f_{M,i}$.

The survival curves in fig 1 include all known antibody-posit infected children. All those born less than 18 months previously h virological evidence of infection irrespective of whether AIDS h developed. There is therefore no selection bias in favour of children.

The sensitivity of HIV-ISS, hyper-IgG, A, M, and low T4/T8 6 and 12 months as predictors of infection (table V) were taken fr the fig 1 survival curves. To take account of the 2 mont persistence built into the definition of HIV-ISS, the sensitivity HIV-ISS was the probability of a first occurrence by 4 and months, respectively—eg, $S_{M,10}$. Likewise, the estima sensitivity of immunological and combined markers was probability of a first occurrence by 5 and 11 months, respective Specificity is estimated from similar survival curves (not shown) the time to each marker in children who lost antibody, but t proportion remaining antibody positive, $S_{AB,a}$ at each age a, w taken into account. For example, the specificity of low T4/T8 at months was estimated by

$$\Sigma_{a<11} f_{M,a} S_{AB,a}.$$

Results

The 600 children were predominantly white caucasi (92%). 66% of their mothers (mean age 25 years) we married or cohabiting. Many of the children had been bo into deprived circumstances: more than 20% had be adopted, fostered, or institutionalised; 28% had dr withdrawal symptoms at birth; and a further 52% h mothers with a history of intravenous drug use (IVDU). children had congenital syphilis, and 2 had symptomle congenital CMV, though ascertainment of these infectio was not complete. Mean birthweight was 2·85 kg; 17% we premature (born ⩽ 36 weeks). 24% of the cohort we delivered by caesarean section (16% elective, 8 emergency). 5% were breastfed for periods from 1 to weeks (median 2·5). Most mothers were symptom free: on 2·8% had AIDS at delivery or within 3 months aft delivery, and a further 2·2% had generalis lymphadenopathy.

64 children were known to be infected: AIDS h developed in 18, of whom 9 had died (table I); there was HIV-related death; 27 children were known to be infecte based on persistence of antibody beyond 18 months; and were antibody positive, aged less than 18 months, b considered infected following virus isolation or antige detection. Among the 353 who lost antibody, 10 had at lea one positive virus culture. The natural history of this grou differed substantially from that of antibody-positiv infected children and will be described separately. 9 deat were not regarded as HIV-related, of which 8 were children of indeterminate HIV infection status. 4 died fro respiratory complications associated with prematurity, an 3 with sudden infant death syndrome (SIDS). 1 child die with meningococcal meningitis at 9 months during a loc outbreak. An antibody-negative child died at 32 mont from tracheobronchitis and streptococcal septicaemia.

Length of follow-up and compliance

Of the 600 children, 380 had been followed for at least year, 216 for more than 2 years, and 100 for more than years. 419 children had been born at least 18 months befor the date of analysis, but the infection status of 47 (11·2%) c these was not known (table I) because they were antibod positive and under 18 months of age when last tested. O these 47 children, 5 had died from non-HIV-related cause

TABLE I—INFECTION STATUS AT LAST FOLLOW-UP

—	Entire cohort	Born ≥18 mo before recruitment
Indeterminate	183 (8)	47 (5)
Follow-up < 1 mo	89 (5)	27 (3)
Follow-up > 1 mo	94 (3)	20 (2)
Lost antibody	353 (1)	324 (1)
HIV-infected	64	48
AIDS	18 (9)	16 (8)
HIV-related death, ab + ve < 18 mo	1 (1)	1 (1)
Persistent antibody > 18 mo	27	27
Antibody positive < 18 mo with virus or antigen	18	4
Total	600	419

No of deaths in parentheses; ab = antibody.

and a further 22 had not been seen since 1 month of age, the parents having moved away shortly after their birth. Any bias was therefore likely to be restricted to the remaining 20 children (4·8% of those born more than 18 months earlier). In the first year of life, the median time between examinations was 2·6 months (5th and 95th centiles, 0·6 and 4·4 months), 3·0 (1·1, 6·7) in the second year, and 4·8 (1·2, 11·9) thereafter.

Treatment

Treatment was less likely to be started in children without AIDS, especially in the first 12 months of life. Of the 49·1 total years of observation of infected children younger than 12 months old without AIDS, only 4·0% were spent under zidovudine treatment, and only 4·1% (partly overlapping) with immunoglobulin (IG) therapy. After the first year, this had increased to 16% for zidovudine and 46% for IG therapy. By contrast, after the onset of AIDS, 66% of the observation time was during zidovudine treatment, and 79% during IG therapy.

Perinatal findings

None of the clinical findings in the perinatal period discriminated between infected and uninfected children. There was no excess of congenital abnormalities in the infected children, and HIV dysmorphic syndrome was not seen. In regression analyses, mothers' IVDU was significantly related to gestation, birthweight, and head circumference, and SD scores for birthweight and head circumference (SD head circumference score $X_3^2 = 8{\cdot}4$, $p = 0{\cdot}04$; all other indices $X_3^2 > 19$, $p < 0{\cdot}0003$). Children with drug withdrawal symptoms were born an average 6 days earlier, were 325 g lighter, had a 7·5 mm smaller head circumference, and had lower SD scores (by 0·57 points on birthweight and 0·48 on head circumference) than children born to mothers with no history of IVDU. Children with no drug withdrawal symptoms but whose mothers had used recreational drugs in the final 6 months of pregnancy were intermediate on all these indices, whereas children of former drug users did not differ significantly from those born to women who had no history of IVDU. There were, however, no statistically significant effects of infection status, and for all indices the lower 90% confidence limit for the difference between infected and uninfected children was less extreme than the mean effects associated with drug withdrawal. In further analyses of infected children who were followed for 18 months, controlling for centre and drug status, there was a tendency for those who had acquired AIDS to be born earlier, lighter, and smaller for dates than those who had not, although these effects were not statistically significant.

TABLE II—SIGNS AND SYMPTOMS ASSOCIATED WITH HIV INFECTION

Sign/symptom	Infected pre-AIDS	Presumed uninfected	Rate ratio	p
Oral candidosis*	13	2	51·5	<0·001
Parotitis	7	2	28·0	<0·001
Petechiae	2	1	15·1	0·02
Eczema	12	33	2·9	0·002
Unexplained fever	9	11	6·5	<0·001
Diarrhoea†	20	47	4·0	<0·001
Bronchitis/bronchiolitis	3	28	0·8	0·6
Gram-negative septicaemia/ septicaemia > 1 mo duration	7	0	..	<0·001
Gram-negative-pneumonia	4	2	15·4	0·001
Other pneumonia	13	21	5·3	<0·001
Chronic otitis media	6	18	2·5	0·03
Chronic purulent rhinitis	10	44	1·8	0·06
Encephalopathy	1	0	..	0·1
Other abnormal neurology*‡	2	5	3·0	0·2
Failure-to-thrive§	2	3	4·9	0·08
Lymphadenopathy*¶	33	44	9·6	<0·001
Hepatomegaly*	37	54	9·5	<0·001
Splenomegaly*	27	16	17·4	<0·001
Hyper IgG‖	40	8	90·8	<0·001
Hyper IgA‖	16	5	35·0	<0·001
Hyper IgM‖	10	5	20·5	<0·001
Low T4/T8**	32	15	22·0	<0·001
No of children	64	343	..	..
No of years of observation	102·5	753·1	..	..
No of examinations	441	2566	..	..

No of children with at least one episode for each sign and symptom.
*On 2 consecutive examinations at least 2 mo apart.
†Persisting > 21 days.
‡Abnormal reflexes or gait, seizures, paresis.
§Fall of > 1·0 in weight for age SD score to < −2·5, or > 0·5 to < −3·0.
¶On at least 2 sites, bilaterally.
‖> 125% of upper reference level for age; in 2 consecutive samples at least 1 mo apart.
**In 2 consecutive samples at least 1 mo apart; below reference level R—R = 1·2–A/100 if 0 < A < 20, and R = 1·0 if A ≥ 20 where A is age in months.

Signs and symptoms associated with HIV infection before onset of AIDS (table II)

The incidence rates show that oral candidosis and parotitis were highly discriminating indices, occurring many times more often in HIV-infected than in presumed uninfected children. Septicaemia and pneumonia with gram-negative bacteria were also strongly associated with HIV infection, although only 4 of the 11 episodes contributed to a subsequent disagnosis of AIDS. Encephalopathy, other neurological abnormalities, and failure-to-thrive were uncommon in the pre-AIDS period; all were associated, but not significantly, with infection. Although there was a highly significant statistical association between many of the signs and HIV-infection status, only a few were specific. For example, of the 67 children with eipsodes of non-cryptosporidium diarrhoea as many as 47 (70%) were uninfected. Lymphadenopathy, hepatomegaly, and splenomegaly were common findings in infected children, but, as evidenced by the low rate ratios, were relatively non-specific on their own, as were eczema, fever, rhinitis, otitis, and pneumonia (except when associated with gram-negative bacteria). By contrast, there was a high incidence of abnormal immunological indices; they discriminated well between uninfected and pre-AIDS infected children, occurring between 20 and 90 times more often in infected children. That bronchitis and bronchiolitis

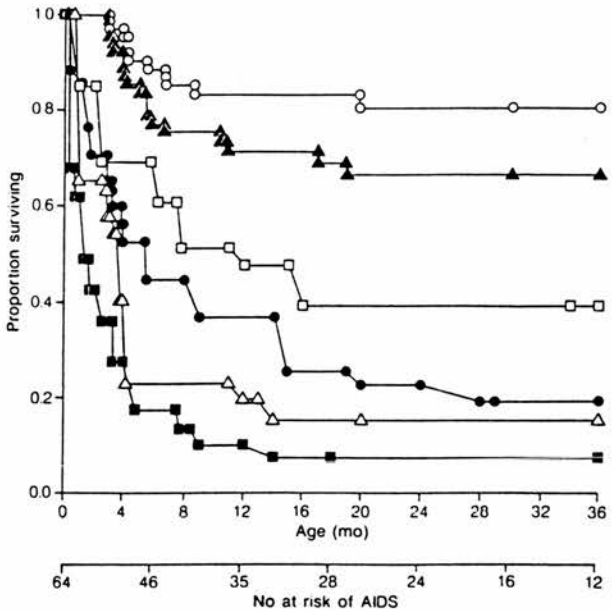

Fig 1—Non-parametric survival curves for 64 antibody-positive infected children.

Time to HIV-related death (○), to AIDS (▲), to low T4/T8 (□), to HIV-ISS (●), to hyper-IgG, A, M, (△), and to the first of any of these features (■).

were not associated with HIV infection suggests that the other results were unlikely to be due to more frequent examinations of children who were already becoming ill.

Progression of disease in infected antibody-positive children

Signs and symptoms in infected and uninfected children are shown in table II. Of the more specific symptoms, such as septicaemia, pneumonia, parotitis, or petechiae, none preceded HIV-ISS; the exception was 1 child in whom septicaemia preceded HIV-ISS by 4 months.

Of the 64 antibody-positive infected children, 30% presented with AIDS within 6 months of age, or with oral candidosis followed rapidly by AIDS. Fig 1 gives the estimated survival curves for time to AIDS onset and for time to HIV-related death. Separate curves for time to HIV-ISS, to hyper-IgG, A, M, and to low T4/T8 are also shown. By 12 months of age, an estimated 17% die of HIV-related causes, 29% have AIDS, 52% low T4/T8 ratios, 73% HIV-ISS, and 81% hyper-IgG,A,M. Altogether, 64% will have shown some feature of HIV disease by 3 months, 83% by 6 months, and 90% by 12 months. Further progression to any of these end-points slowed substantially during the following 2 years. No child got AIDS after 20 months of age (see fig 1 for number at risk).

27 infected children survived without AIDS to 18 months; in 9, no symptoms developed in the average 29 months of observation. In the remaining 18 (67%), followed for an average 30 months, lymphadenopathy, hepatomegaly, and/or splenomegaly developed at a median age of 6 months. In 11 children (41%), these symptoms persisted during follow-up, but in 7 (26%) the symptoms resolved within 5–25 months, in most cases without treatment. Continuing symptoms were prolonged periods of at least two of lymphadenopathy, splenomegaly, and

TABLE III—PRECURSORS OF AIDS IN FIRST 12 MONTHS

—	AIDS/HIV death within 12 mo	No AIDS/HIV death within 12 mo	Rate ratio	p
Oral candidosis	11	2	35·7	<0·001
LHS*	7	18	1·8	0·1
Hyper-IgG	5	23	0·9	0·7
Hyper-IgA	2	11	1·7	0·2
Hyper-IgM	4	4	5·8	0·009
Low T4/T8	7	14	2·2	0·1
No of children	17	35	..	..
No of years of observation	7·2	35·0	..	..
No of examinations	130	465	..	..

*Any 2 concurrently, of lymphadenopathy, hepatomegaly, and splenomegal persisting for 2 mo.
Other definitions as in table II.

hepatomegaly, and in some children episodes of parotitis petechiae, septicaemia, unexplained fever, and pneumonia Resolution of symptoms was seldom complete, and persistent lymphadenopathy tended to remain. By contrast immunological disorders rarely resolved. No child recovered normal T4/T8 levels once the ratio had fallen below reference levels (see table II). Hyper-IgG resolved in only 1 instance, though this feature could have been concealed in some of the 19 children who were receiving IC therapy. Hyper-IgA and hyper-IgM were generally transient.

Fig 1 shows that hyper-IgG,A,M is usually the first feature of disease, followed by HIV-ISS, low T4/T8 ratio and finally AIDS. Most of the infected children have so far shown a progression consistent with this order. Hyper-IgG,A,M, was the first or equal first sign in 74%. The relative timing of the low T4/T8 ratio was more variable: it came after the onset of AIDS in 44% of those in whom AIDS developed, and predated HIV-ISS in 20% of those in whom pre-AIDS HIV-ISS or low T4/T8 were recorded.

TABLE IV—CLINICAL SPECTRUM OF AIDS OR HIV-RELATED DEATH: INDICATOR DISEASE AND PROGRESSION

Initial AIDS indicators (age at onset, mo)	Progressing to (age, mo)	Age last seen(mo)
PCP (4)	..	4*
PCP, FTT, encephalopathy (5)	..	5*
PCP (5)	FTT, encephalopathy (13)	31
PCP (3)	BCG-itis (10)	24
PCP (3)	..	10
PCP (3)	..	21
CMV, encephalopathy (5)	..	6*
CMV (3)	..	3*
CMV (2)	FTT, PCP, encephalopathy (5)	9*
Septicaemia, LIP (10)	FTT (15), PCP, encephalopathy (19)	19*
Pneumonia (10)	..	26
Pneumonia (3)	FTT (7)	8*
Septicaemia (5)	..	17
LIP (3)	..	4*
LIP (5)	Encephalopathy (24)	30
LIP (4)	..	4*
Cryptosporidium (17)	..	31
Cryptosporidium (18)	FTT (9), pneumonia (34)	34
None	Oral candida (3), SUD (6)	6*

*Died.
PCP = *Pneumocystis carinii* pneumonia; CMV = disseminated cytomegalovirus infection; LIP = lymphoid interstitial pneumonitis; FTT = failure-to-thrive; SUD = sudden unexplained death.

Precursors of AIDS onset in the first 12 months

The 17 children who got AIDS or died of HIV-related illness in the first 12 months are compared with the 35 antibody-positive infected children who survived to 12 months without AIDS (table III). Oral candidosis was highly predictive of imminent AIDS, but combinations of lymphadenopathy, hepatomegaly, and splenomegaly were only marginally associated with progression to AIDS. Immunological indices were less strongly predictive of AIDS than they were of HIV infection, with the exception of hyper-IgM.

In a separate analysis, of the children who got AIDS, 10 out of 13 (77%) had at least one positive antigen test in the pre-AIDS period (14 of a total 32 tests were positive), compared with 20 out of 27 (74%) of antibody-positive infected children who did not get AIDS and who were followed for over 18 months (74 of a total 184 tests).

Clinical features of AIDS

Table IV shows the indicator diseases and clinical course in the 19 children who got AIDS or who died of HIV-related causes. Within the first 6 months, 15 had AIDS or died, and significant HIV-ISS and hyper-IgG had already developed in the remaining 4. 1 child died unexpectedly at 6 months: oral candidosis had developed more than two months previously. (Because of the poor outcome associated with oral candidosis in this study, this death was assumed to be HIV-related, and has been included with the 18 AIDS children in the analyses). 2 of the children with encephaiopathy had microcephaly; diagnosis roughly coincided with onset of AIDS. Failure-to-thrive was seen in 4 out of 6 of those with encephalopathy compared with 2 of 13 of those without this disorder (Fisher's two tailed, $p = 0{\cdot}05$). 1 of the 2 children with encephalopathy but normal growth had a brain lymphoma and may have been atypical. Of those with failure-to-thrive but no encephalopathy, 1 was reportedly malnourished and the other had cryptosporidium diarrhoea. A further child, without AIDS, had both failure-to-thrive and abnormal neurological signs—features which were rare in pre-AIDS children (table II).

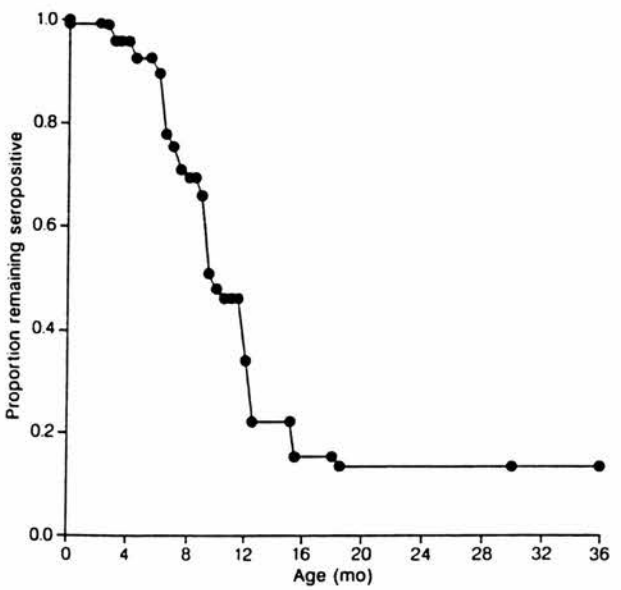

Fig 2—Survival estimates of the proportion of the cohort remaining antibody positive.

Data adjusted for HIV-related deaths.

TABLE V—SENSITIVITY AND SPECIFICITY OF CLINICAL AND IMMUNOLOGICAL INDICES AS MARKERS OF INFECTION

Index	Sensitivity (%)		Specificity (%)	
	6 mo	12 mo	6 mo	12 mo
Hyper-IgG, A, M	77	77	97	97
Low T4/T8	31	49	99	98
Hyper-IgG, A, M or low T4/T8	79	82	96	95
HIV-ISS	47	63	97	94
HIV-ISS, hyper-IgG, A, M, or low T4/T8	83	90	94	90
Two of HIV-ISS, hyper-IgG, A, M, or low T4/T8	48	69	100	100

9 of the 18 children with AIDS have died, 5 within a month of diagnosis. Of the 9 remaining alive, 5 (all of whom had AIDS indicator diseases for which there is moderately effective treatment) had lymphadenopathy or hepatomegaly when last seen. 2 children with AIDS became seronegative at or shortly after the onset of AIDS; both have died.

Loss of antibody

In children who lost antibody, there were differences of up to 5 months between centres in the mean age of the first negative antibody test. The differences were much larger than could be accounted for by variation in the scheduling of tests, and were probably due to the different antibody test systems used. However, most samples were tested by both ELISA and western blot, and no child was persistently positive by one system while remaining negative by the other.

Fig 2 gives the survival curve estimate of the proportion who remain antibody positive at each age, and is based on the 520 cohort members with an antibody determination after their birthdate. Antibody loss is assumed to have occurred after the last positive test but before the first negative test, if there was one. Children who died with AIDS/HIV are assumed to be antibody positive at the time of analysis, and the 2 children with AIDS who subsequently lost antibody are assumed to have remained seropositive. With these adjustments, the curve reaches an asymptote at 13·4% (95% confidence interval [CI]: 9·7–17·1%), and this is an estimate of the vertical transmission rate (see below). Of those who will lose antibody, an estimated 10·2% lose it after 15 months, and 2·5% after 18 months.

343 children have been followed for a total of 390 years after their first negative antibody result, including 80 followed beyond their 3rd birthday; none has yet developed AIDS or persistent immune deficiency, or has again developed persistent antibody or antigen.

Recognition of infection in antibody-positive children, without definitive laboratory diagnosis

The sensitivity and specificity of signs and symptoms as predictors of infection in antibody-positive children are shown in table V. Hyper-IgG,A,M, was the most sensitive indicator, identifying at least 77% of infected children by 6 months, with an estimated false positive rate of 3% in uninfected children who are still seropositive. Combinations of hyper-IgG,A,M, low T4/T8, or HIV-ISS increased sensitivity at 6 months but lowered specificity. A combination of any two of the three variables would

recognise 48% of infected infants by 6 months. None of the 325 children who lost antibody and who were followed for over 12 months met this criterion in the first 12 months. The specificity is therefore estimated to be 100% and with this sample size is unlikely to be less than 99·1% (lower 90% confidence limit).

Vertical transmission rate

Of 419 children born 18 months or more before June 15, 1990, 372 were of known infection status and of these 48 were infected (table 1). This represents a transmission rate of 12·9% (95% CI 9·5–16·3%), which agrees closely with the estimate of the surviving fraction of 13·4% in fig 2. There were no statistically significant differences in transmission rates between centres, nor were there changes over time. Of the 353 children who lost antibody, virus isolation had been attempted in 163; in 10 (6·1%), positive results were obtained at least once. With the exception of 1 child in whom a static encephalopathy uncharacteristic of HIV developed following a Reye-like syndrome,[12-15] these 10 children did not seem to differ clinically or immunologically from the other 343 children who lost antibody. 1 child had hyper-IgG, another a low T4/T8 ratio, and a third lymphadenopathy and hepatomegaly. However, these signs all resolved within a few months, and taken together they represent an incidence of the same order as would be expected in children who lost antibody. In 4 children the initial positive culture was confirmed on subsequent samples by repeat virus culture and/or polymerase chain reaction (PCR). But in the remaining 6, a total of eighteen attempted cultures were negative, and laboratory error or contamination cannot be completely ruled out. The data do not, therefore, support an estimate greater than 4/163 (2·5%, 95% CI 0·7–6·3%).

Discussion

As a description of the natural history of HIV-1 infection up to diagnosis of AIDS in the first 3 years of life, we believe that the ECS is substantially unbiased by treatment. While confirming the wide spectrum of disease found by other investigators,[1-3,7,9,16-23] the ECS provides additional information on the timing of initial symptoms, and the frequencies of clinical and immunological features.

Birthweight, gestation, head circumference at birth, or birthweight adjusted for gestation did not discriminate between infected and uninfected children; this also confirms earlier results.[7,11] The lower birthweight of children born to women with AIDS or with symptomless HIV infection than of those born to seronegative women reported in two African-based studies[22,24] is more likely to be due to differences in the mothers' socioeconomic or health circumstances than to interuterine infection, since these findings have not been seen in New York[25] or Scotland.[26]

Our findings show that without a definitive diagnosis, immunological and clinical signs can be regarded as predictors of HIV infection in children under 18 months old. Although several signs and symptoms are associated with HIV infection, the ECS shows that many are not specific enough to be useful markers either of infection or of the development of AIDS. Otitis media, rhinitis, non-cryptosporidium diarrhoea and unexplained fever were all poor indicators of infection compared with hyper-IgG,A,M, which identified 77% of infected children at 6 months with 97% specificity. In a population of whom 15% are infected, this implies a positive predictive value of on 80%, and may not therefore be an indication for therapi with side-effects. A combination of any two of hype IgG,A,M, low T4/T8 ratio, or HIV-ISS was highly specif and could be used as a criterion for treatment or inclusion i a therapeutic trial. However, these findings should not b applied to children whose mothers are not known to b positive, because immunological signs could have very lo predictive value in the general population. Moreover, ou findings would not be applicable in countries wher hyper-IgG, lymphadenopathy, and hepatosplenomegaly due to chronic infections are common.[27] The relativ frequency of AIDS indicator diseases in the ECS is simila to earlier reports.[7,20,28] The possible association betwee failure-to-thrive and encephalopathy would point to central nervous system mechanism for failure-to-thrive tha is different from the opportunistic diarrhoeal infections tha probably underlie failure-to-thrive in African studies.

Our findings have implications for clinical managemen and for the design of clinical trials. Firstly, treatment wil have to be started very early—within the first 2 or months—to prevent the early onset of AIDS in about 30% of infected children.[7] Except in laboratories with high expertise, such an early diagnosis would be hard to achieve a present. Also, during the second and third years of life, th progression of HIV disease seems to be slow, with more children improving than deteriorating. Long-term treatment of symptom-free children or those with mild symptoms may be necessary to show any benefit; this no only creates difficulties in the design of trials for treatments with side-effects, but also raises doubts about earlier, open therapeutic studies that attributed to treatmen improvements that might have occurred spontaneously.[29-30]

Our 13% estimated transmission rate is lower than rates published in all but one study,[31] which was a subset of the ECS. It is possible that a higher frequency of risk factors such as breastfeeding or mothers' clinical condition could account for these differences. However, estimates in many earlier studies may have been biased upwards. For example, in one study, virus cultured from cord blood was taken as proof of infection.[22] In a study citing 39% transmission, 47% of the cohort had been lost to follow-up;[24] and those remaining may have been more likely to have been ill. A 29% rate was cited in a New York Study.[32] However, that analysis included antibody-negative children only if they had been followed for 15 months, whereas infected children were included with shorter follow-up. In studies in which some children are recruited retrospectively, the requirement that the mother should be seropositive may be insufficient to prevent a bias towards selective recruitment of sick children. A further requirement is that collaborating centres report all children born to seropositive mothers. This type of bias may have occurred in the Italian Multicentre Study[23] (33% transmission), which is register based; in the French Study,[7] which has 51 collaborating centres (27% transmission); and also in earlier reports from the ECS.[10,11] The requirement for inclusion in the transmission rate that children should have an antibody test after 18 months[11] rather than have a birth date 18 months earlier also biases estimates upwards because parents of children who lose antibody earlier are less likely to bring the children back for continuing follow-up. Even if all the children who were lost to follow-up were infected, the transmission rate would still only be 23%.

Deaths in children of indeterminate infection status, of which there were 7 in the ECS, also make the calculations of

transmission rates difficult. The mortality rates we found are higher than the 1/600 SIDS[33] and the 5/1000 neonatal mortality rates[34] in the UK, though the social deprivation of our cohort must be taken into account. Even if all these deaths are included as HIV related, the estimated transmission rate would only be increased by 1·2%. The 24% transmission rate cited in an earlier report from the ECS[11] included the children who lost antibody but in whom virus was detected. However, virus isolation in healthy antibody-negative children reported by the ECS[35] and elsewhere[3] is difficult to quantify since laboratory error cannot be ruled out in every instance. Our results do not suggest that this finding occurs in more than 2·5% of those who remain antibody negative and free of immune deficiency. Further reports of viral DNA detected by PCR in antibody-negative children,[36] unconfirmed by viral isolation, are even more difficult to interpret[37] and require further evaluation.[38]

We have not yet seen any child first lose antibody and then become persistently antibody positive or show any immunological or clinical signs of infection. This failure to duplicate earlier reports of seroreversion in 2-year-old children,[39] despite follow-up of some 80 children beyond age 3, not only is reassuring, but also implies that infection of children through household contact must be rare since it has not occurred once in 390 person-years observation of antibody-negative children.

The mode of vertical transmission remains unknown. The early onset of clinical and immunological features in 80–90% of infected children points to intrauterine transmission rather than transmission at delivery. However, rapid onset of HIV-related illness has also been reported in children infected by transfusion at birth,[40] with clinical illness in 1 child as early as 4 months.[20]

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Mortality in hereditary antithrombin-III deficiency—1830 to 1989

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To determine whether antithrombin-III (AT-III) deficiency leads to an excess mortality, we studied 171 individuals from ten families with a proven hereditary deficiency. 73 were classified as certainly deficient either by direct measurement of AT-III concentration or by mendelian inheritance patterns. 98 individuals had a high probability (0·5) of deficiency. The 64 deaths recorded did not exceed those expected for the general population adjusted for age, sex, and calendar period. We suggest that a policy of prophylactic anticoagulation for patients with AT-III deficiency cannot be recommended.

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Introduction

Hereditary antithrombin-III (AT-III) deficiency is an uncommon autosomal disorder that is associated with a tendency to venous thromboembolism in heterozygous individuals.[1,2] The severity of venous thrombosis ranges from superficial thrombophlebitis to pulmonary embolism but the risk of severe thromboembolism in AT-III deficient individuals is largely unknown.

The decision to anticoagulate symptom-free AT-III deficient individuals prophylactically is therefore difficult and a randomised trial of anticoagulant treatment against placebo is unfeasible since the disorder is rare and a long follow-up would be required to give a definitive answer. Furthermore, the risks of long-term anticoagulant treatment are substantial.[3]

To study the natural history of AT-III deficiency, we compared mortality in AT-III deficient families with that of the general population. If the mortality were lower in the general population, one could estimate the potentially beneficial effect of anticoagulant treatment.

Subjects and methods

Study population

All Dutch families, with a member known to be AT-III deficient and who was under the care of the Departments of Haematology of the University Hospitals of either Leiden or Amsterdam, were eligible for study. Diagnosis was based on AT-III antigen concentrations less than 75% of normal, in the absence of heparin treatment, chronic liver disease, or nephrotic syndrome. Only families with 2 or more deficient individuals were studied, to avoid inclusion of patients with an acquired deficiency. Furthermore, we included only families with at least one symptomatic deficient individual.

In all families, non-deficient individuals were excluded and for untested family members information from relatives was used to assign a probability of deficiency. Since AT-III deficiency is an autosomal disorder, some untested individuals can be taken as heterozygous for AT-III deficiency—these family members have passed on the affected gene from common ancestors to deficient individuals. Mendelian probabilities can be assigned to all individuals in a pedigree. We restricted our study population to those who were deficient with certainty or had a probability of deficiency of 0·5. We rejected the possibility of recent mutations, which may theoretically have taken place in smaller pedigrees, because of the low frequency of AT-III deficiency. This method requires complete pedigrees. Since 1809 it has been mandatory by law in the Netherlands to report all births and deaths to the municipal registries. All information in this study has been verified by reference to these municipal and national registries.

Analysis

The mortality of the study population (observed) was compared with the general population adjusted for age, sex, and calendar period (expected). The ratio of observed to expected number of deaths (O/E) is the standardised mortality ratio (SMR). The expected mortality is derived by multiplying the total number of years lived by the study population in each calendar period, and for each age and sex category, by the mortality rates (age and sex specific) of the general population for each calendar period. Confidence limits for the SMR are based on a Poisson distribution for the observed number of deaths.[4]

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SHORT COMMUNICATION

Increased expression of the CD45RO (memory) antigen on T cells in HIV-infected children

Karin S. Froebel, Kathleen V. Doherty, James A. Whitelaw, Rosemary A. Hague*, Jacqueline Y. Mok* and A. Graham Bird

Expression of the CD45RO putative memory cell antigen on CD4 (helper) and CD8 (cytotoxic/suppressor) lymphocytes of children born to HIV-infected women was investigated using the UCHL1 antibody. Significantly raised numbers of CD45RO+ CD8 lymphocytes were found in all nine of the infected children compared with uninfected and control children. Expression of CD45RO on CD4 lymphocytes was variable; absolute numbers were not increased, although the percentage was increased in four out of nine infected children. All the infected children except two (who had comparatively low numbers of CD45RO+ CD8 cells) were clinically well, which suggests that an increase in CD45RO+ CD8 cells may be indicative of a functionally active immune response against HIV.

AIDS 1991, 5:97–99

Keywords: Paediatric HIV infection, leucocyte common antigen, cell-mediated immunity, phenotypic markers.

ntroduction

IIV destroys the immune system by infecting and grad-ally eliminating, both numerically and functionally, the elper T-cell subpopulation of lymphocytes. One of the rst functional immune responses to decline is the mem-ry T-cell response, manifest by a loss of skin test respon-iveness to recall antigens, and a fall in the *in vitro* pro-ferative response to these same antigens. The CD45RO ntigen, recognized by UCHL1 antibody, is a 180 kD vari-nt of the leucocyte common antigen, resulting from al-ernative splicing of the mRNA, and is expressed on a pro-ortion of T cells, monocytes and polymorphonuclear ells [1]. In the peripheral T-cell population the CD45RO ariant is thought to be a marker for antigenically primed r memory cells [2]. Ontologically, CD45RO expression n T cells is minimal at birth, and rises during the first ew years of life, presumably as a child is exposed to new ntigenic challenges, reaching a level of between 40 and 0% by 15 years of age [3].

ecause of the disturbance of the memory T-cell pop-lation in HIV infection, we decided to investigate the expression of CD45RO on T lymphocytes in infants and children born to HIV-positive women. There are now 103 such children in Edinburgh, of whom eight are confirmed as HIV-infected and are still alive. We analysed both the CD4 and CD8 T-cell subpopulations, and our results have shown that, rather than there being an absence of these putative memory cells, the development of CD45RO+ cells is accelerated in children known to be infected with HIV, reaching levels far in excess of those found in un-infected children or in infected adults.

Subjects and methods

Subjects

The subjects were children born to HIV-infected women who were being regularly followed up at the Infectious Diseases Unit at the City Hospital, Edinburgh, Scotland, UK. In addition, one sample was taken from a 75-month-old child who had been infected through a blood trans-fusion. Cord blood samples were obtained from the

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mpson Maternity Hospital both from babies born to IV-infected women and from normal deliveries. Asymp- matic children were confirmed as being HIV-infected nly if their serum HIV antibody test remained positive 3 months after delivery. One child of 15 months who ad symptomatic disease and who was HIV-antibody- and rus-culture-positive was included in the 'infected' group. l other children younger than 18 months were con- dered to be 'indeterminate' with regard to HIV infec- on. Control samples were taken (1) from children born HIV-positive women who were over 18 months and ronegative or who had seroreverted (defined as 'pre- med uninfected'), and (2) to age-match the older pa- nts, from selected routine samples sent to the Depart- ent of Paediatric Haematology, Edinburgh Royal Hos- tal for Sick Children. An analysis of variance was car- ed out on the data obtained from the normal control ildren and the presumed uninfected children which owed that there was no significant difference between ese groups in the percentage of CD45RO+, CD3+ mphocytes ($f = 0.372$ with 27 d.f., data not shown). We erefore assumed that CD45RO expression on CD4 and D8 subsets would also be similar, and pooled the data om these two groups for subsequent analysis.

ethods

mphocyte subpopulations

hole EDTA blood (100 µl) was incubated with 5 µl each fluorescein isothiocyanate (FITC)- conjugated UCHL1 tibody (Dako, High Wycombe, UK) and phycoerythrin E)- conjugated CD3, CD4 or CD8 antibody (Becton- ckinson, Oxford, UK), and the samples were analysed a Becton-Dickinson FACScan. Analysis was performed a lymphocyte gate defined both by forward and side atter characteristics and also on phenotypic evaluation ecton-Dickinson 'leucogate').

cause of the age variation in children in both the num- r of circulating lymphocytes and in the percentage of 045RO-bearing cells, the data were analysed in four age nds: cord blood, 0– 17 months, 18–36 months and –80 months. Results for patients and controls in each e band were compared using a Mann–Whitney U test.

Results

The results are shown in Table 1 and Fig. 1. In 20 cord-blood samples and 22 samples from children under 17 months of age there were no significant differences in CD45RO expression on CD3 cells in the 'at risk' children compared with normal control children (data not shown).

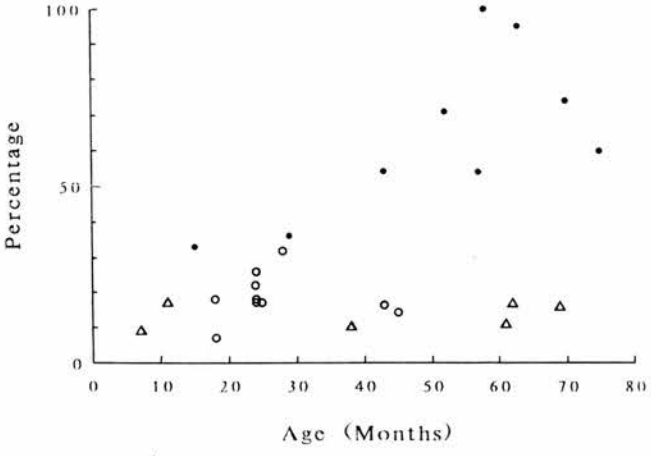

Fig. 1. Expression of CD45RO on CD8+ T cells in normal controls (△), presumed uninfected children of HIV-infected mothers (○), and HIV-infected children (●).

In children over 18 months old the percentage of CD45RO+ CD4 cells was higher in infected children compared with the group of uninfected 'at risk' and normal children. However, when the results are expressed as absolute numbers of CD4 cells to take into account the lower number of CD4 cells in the infected children, the number of CD45RO+, CD4+ cells was not significantly different in the two groups (Table 1).

In the CD8 subset, both the percentage and the absolute number of CD45RO+ cells were significantly raised in HIV-infected children compared with the uninfected 'at risk' and control children (Table 1, Fig. 1). In all of seven infected children over 3 years the percentage of CD45RO+ CD8+ cells exceeded the upper limit of the normal range [3].

ble 1. Expression of CD45RO on CD4 and CD8 cells in children between 18 and 80 months of age.

			CD45RO expression			
			CD4		CD8	
e (months)	HIV status	No.	%	n/µl	%	n/µl
36	Infected	2†	35	330	35*	490*
	Uninfected/control	9	15	410	18	180
80	Infected	7	56**	280	71**	1370**
	Uninfected/control	6	31	345	16	125

ents significantly higher than controls (Mann–Whitney U test): *$P < 0.05$; **$P < 0.01$. †Includes one 15 months-old patient (see text).

Discussion

Our results show abnormally enhanced expression of the CD45RO antigen on peripheral blood T cells of HIV-infected children. The increased expression occurs primarily in the CD8 (suppressor/cytotoxic) subset of T cells, with all of the infected children having results above the normal range, and in two out of nine cases over 95%. Increased percentages of CD4 (helper) T cells were observed in four out of nine infected children. Taking into account the differences in absolute numbers of CD4 and CD8 cells in the HIV-infected and HIV-uninfected control children, the differences in the CD45RO+ CD8+ subpopulation becomes even more marked, whereas the differences in the CD45RO+ CD4+ subpopulation are reduced to the extent that only one patient has a higher level than that observed in uninfected children.

Hayward *et al.* [3] first showed that expression of CD45RO on both CD4+ and CD8+ lymphocytes increases over the first years of life from 2 and <0.5%, respectively, at birth to 45 and 35%, respectively, at 6 years of age, rising to a maximum of 55 and 48%, respectively, by 20 years. This finding together with functional studies have led to the conclusion that CD45RO is a marker for memory T cells in both the proliferative (helper) and cytotoxic subsets [2,4,5]. Whether or not these cells are functionally active in the HIV-infected children and, if so, whether they are HIV specific, has still to be determined.

All but two of the children in this study have remained asymptomatic, and these two (the children aged 15 and 4 months in Fig. 1) have relatively fewer CD45RO+ CD8 cells than those who are asymptomatic, suggesting that CD45RO+ CD8 cells may be functionally important in suppressing viral replication. The extent of CD45RO expression observed in the HIV-infected children has not to our knowledge been described previously in peripheral blood T cells, and the immune processes which drive the majority of CD8 cells into 'memory' cells are unknown.

Van Nossel *et al.* [6] have recently reported that expression of another putative marker of memory cells, the CD29 antigen, falls in HIV-positive homosexual men. Our own preliminary observations on HIV-infected adults show no significant differences in CD45RO+ CD4 or CD8 cells between groups of patients with normal or reduced absolute CD4 counts (unpublished observations). The reason for the apparent discrepancy between their results and those reported here is unclear. One possible explanation is that the CD8 cell-mediated immune response to HIV differs in children infected perinatally and in adults. Further phenotypic studies are in progress which should clarify this point.

Expression of CD45RO on CD4 cells was variable, and there was no clear relationship between CD45RO expression on CD4 cells and HIV infection. However, it is of interest to note that, in the HIV- infected patients in a situation of a falling CD4 count, the CD45RO+ cells appeared to be preferentially retained in the circulation.

The function of the CD45 leucocyte common antigen molecule is still poorly understood, but it may serve as a regulatory molecule of cell activation [7], and it may be that the different isoforms have different regulatory functions. This study has shown a clear phenotypic difference in the CD8 lymphocyte subpopulation between HIV-infected and uninfected children. The demonstration of accelerated expression of CD45RO+ CD8+ cells may be a useful early indicator of HIV infection in at-risk children. Evidence of this, however, has been hindered by the very low level of vertical HIV infection in the Edinburgh children cohort and awaits the results of an extensive longitudinal study which is now under way. Whether the enhanced expression of CD45RO on CD8 lymphocytes represents a functionally active subpopulation of immune cells or whether it represents an HIV-induced aberration of the normal differentiation pathway also remains to be established.

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HLA ANTIGEN FREQUENCIES IN CHILDREN BORN TO HIV-INFECTED MOTHERS

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SUMMARY

Tissue-typing for HLA-A, B, and DR antigens was carried out on 53 babies, 47 of them unrelated, born to mothers known to be HIV-infected from intravenous drug usage or sexual contact with drug users. These babies were followed up to assess whether HLA phenotype was associated with vertical transmission of HIV infection or disease progression. Of the 47 unrelated babies, eight became infected with HIV. The frequency of HLA-DR3 was three times higher in the HIV-positive infants compared to the HIV-negative infants (43 per cent vs 15 per cent) in our study population. Conversely, HLA-A3 was three times less common in the HIV-positive infants (12·5 per cent vs 42 per cent). A comparison of HLA antigens between our study group babies and babies born to healthy mothers unselected for HIV status revealed higher proportions of HLA-B18, B7, and DR2 in the study group. Moreover, the combination, A3, B7, DR2 was four times commoner in our study population relative to controls ($RR = 3{\cdot}9$; $p < 0{\cdot}003$), but was found only in babies who were not HIV infected. The combination A1, B8, DR3, in contrast, was found less often than expected in our study group ($RR = 0{\cdot}39$) and was disproportionately represented amongst the infected babies. We have observed an unexpectedly low (6 per cent) mother-to-infant transmission rate of HIV among prospectively studied intravenous drug users. We speculate that the unusually high ratio of the common antigen combinations (often halotypes), A3, B7, DR2 to A1, B8, DR3 in this population may be contributory.

KEY WORDS HIV Paediatric AIDS HLA disease associations Vertical transmission of HIV

INTRODUCTION

Human immune deficiency virus (HIV) disease is rather variable in course, which may in part be a reflection of individual differences in genetic susceptibility. Several studies have associated HLA specificities with aspects of HIV infection. Examples include HLA-B35 and progress from lymphadenopathy syndrome (CDC stage 3) to AIDS (CDC stage 4) in subjects infected predominantly from intravenous drug use (Smeraldi *et al.*, 1986; Smeraldi *et al.*, 1988); HLA-DR5 with HIV-associated Kaposi's sarcoma (Pollack *et al.*, 1983; Prince *et al.*, 1984), and thrombocytopenia (Raffoux *et al.*, 1987) in homosexuals; and HLA-DR3 with disease progression in haemophiliacs (Steel *et al.*, 1988; Kaslow *et al.*, 1990; Fabio *et al.*, 1990), but not in a predominantly homosexual population where the opposite trend was apparent (Kuntz and Bruster, 1989). In addition to individual specificities, the combination or

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haplotype HLA-(A1, B8, DR3) has been associated with relatively rapid loss of circulating CD4-positive lymphocytes (Steel *et al.*, 1988; Kaslow *et al.*, 1990; Cameron *et al.*, 1991), and possibly with an increased susceptibility to infection (Steel *et al.*, 1988).

One group at risk for HIV infection which has not previously been studied in relation to tissue type is the children of HIV-infected mothers. Although transplacental infection is well documented, not all babies are born infected. In principle, HLA phenotype might influence disease susceptibility *in utero*, or, amongst those affected, influence disease progression in infancy. We therefore carried out a preliminary study to look for HLA associations in such children.

PATIENTS AND METHODS

The subjects were 53 neonates or infants born to mothers known to be HIV-1 positive. The study group was basically part of a wider perinatal transmission study described elsewhere (Mok *et al.*, 1989), although we also obtained samples for tissue typing from five (three unrelated) other Scottish infants known to be HIV positive. Most of the mothers were drug users infected directly from needle sharing, but five were apparently infected sexually by HIV-positive male drug users. Most of the mothers lived in Edinburgh, were Scottish, and shared a social milieu characterized by multiple deprivation. Whenever possible, tissue typing was performed immediately after birth from cord blood; otherwise samples were obtained during follow-up visits.

Infants who remained HIV-antibody positive 15 months after birth and who had symptoms consistent with, or suggestive of, paediatric HIV infection (European Collaborative Study, 1988) were diagnosed as HIV positive. Infants who had become seronegative by 15 months of age, were healthy, and had normal immune function, were presumed uninfected.

Tissue typing for HLA-A, B, and DR antigens was carried out as previously described (Jazwinska and Kilpatrick, 1987). Control data from unselected babies born to healthy mothers were taken from a previous study (Jazwinska and Kilpatrick, 1987).

RESULTS

The HLA antigen frequencies obtained from 47 unrelated babies born to HIV-infected mothers are listed in Tables 1 and 2. The frequencies for the total group under study differed little from those of the control group born to healthy mothers (unselected for HIV status). However, there were marked increases in frequencies of HLA-B18 ($RR=7{\cdot}9$; $\chi^2=7{\cdot}9$; $p=0{\cdot}005$), B7 ($RR=1{\cdot}9$; $p>0{\cdot}05$), and DR2 ($RR=2{\cdot}0$; $\chi^2=3{\cdot}74$; $p=0{\cdot}05$). The associations with HLA-B18 and DR2 were not prior hypotheses, however, and so are not statistically significant after correction for the number of antigens tested.

Comparing HLA antigen frequencies between babies positive and negative for HIV, the most striking difference was in HLA-DR3, which was three-fold higher in the infected infants. Frequencies of HLA-A1 and HLA-A2 were also elevated in

Table 1. HLA Class 1 antigen frequencies in unrelated neonates born to women infected with HIV-1

Specification	% HIV-positive neonates ($n=8$)	%HIV-negative neonates ($n=38$)	% Total neonates ($n=46$)†
A1	50	23·7	28 (39)
A2	75	36·8	41 (52)
A3	12·5	42	37 (32)
A11	0	18	15 (11)
A23	0	0	0 (4·5)
A24	12·5	15·8	13 (17)
A25	0	15·8	13 (4·5)
A26	25	0	4·3 (3)
A28	12·5	2·6	4·3 (4·5)
A29	0	5·3	4·3 (5·3)
A30/31	12·5	5·3	6·5 (5·3)
A32	12·5	7·9	8·7 (3·8)
B5	0	5·3	4·3 (4·2)
B7	37·5	47	46 (31)
B8	25	18	20 (31)
B13	12·5	2·6	4·3 (5)
B14	0	10·5	8·7 (9)
B15	0	7·9	6·5 (10·6)
B16	0	5·3	4·3 (6·8)
B17	25	10·5	13 (8)
B18	12·5	10·5	11 (1·5)
B21	25	0	4·3 (4·5)
Bw22	0	7·9	6·5 (5·3)
B27	0	5·3	4·3 (6·8)
B35	12·5	15·8	15 (14)
B37	0	0	0 (3)
B40	0	10·5	8·7 (11·4)
B44	25	15·8	17 (27)

†Figures in parentheses are the corresponding proportions for control neonates born to healthy mothers unselected for HIV status (Jazwinska and Kilpatrick, 1987).

the infected group, while HLA-A3 was proportionately more abundant in the HIV-negative group.

The rather high frequencies of both B7 and DR2 prompted us to examine the second commonest HLA antigen combination in the general population, A3, B7, DR2, as well as the much commoner A1, B8, DR3 combination which has formerly been implicated in disease susceptibility by others (Table 3). The combination A3, B7, DR2 (which often occurs as a haplotype) was four-times commoner in our study population relative to the controls ($RR=3·9$; $\chi^2=9·1$; $p<0·003$), but was found only in babies who were not HIV infected. The combination A1, B8, DR3, in contrast, was found less often than expected in our study group ($RR=0·39$; $p=0·06$) and was disproportionately represented amongst the infected babies.

Five families were also studied. (One member of each was included in the unrelated series). There were three sets of dizygotic twins, a pair of brothers, and a set

Table 2. HLA Class 2 antigen frequencies in unrelated neonates born to women infected with HIV-1

Specificity	% HIV-positive neonates ($n=7$)	% HIV-negative neonates ($n=39$)	% Total neonates ($n=46$)†
DR1	0	10	8·7 (12)
DR2	43	44	43 (28)
DR3	43	15	19·6 (39)
DR4	29	28	28 (25)
DR5	14	18	17 (13)
DRw6	29	18	19·6 (34)
DR7	29	33	32·6 (26·5)
DRw8	0	2·6	2 (3·8)
DRw9	0	5·1	4·3 (0)

†Figures in parentheses are the corresponding proportions for control neonates born to healthy mothers unselected for HIV status (Jazwinska and Kilpatrick, 1987).

Table 3. HLA antigen combinations in unrelated neonates born to HIV-infected women

Combination	% HIV-positive neonates ($n=7$)	% HIV-negative neonates ($n=38$)	% Total neonates ($n=45$)	% Controls ($n=132$)
A1, B8, DR3	26·8	7·9	11·1	24·2
A3, B7, DR2	0	28·9	24·4	7·6

of three sisters. Of all the twins, only one child was HIV infected and she shared one haplotype with her uninfected sister; the two uninfected twin pairs shared one and two haplotypes, respectively. The brothers, neither of whom were infected, shared one haplotype. Two of the three sisters, all of whom were HIV infected, were HLA identical (A2, A28; B7, B21; DR2, DRw6): one died aged 25 months of HIV wasting syndrome, the other has interstitial pneumonitis but remains well at 5 years of age. The third sister (A24, A28; B7, B16; DR uncertain) shared one haplotype with the other two, and died at 67 months, cachectic after persistent bacterial pneumonia.

DISCUSSION

Early data on mother-to-infant transmission of HIV infection indicated a rate as high as 64 per cent (Scott *et al.*, 1985). However, later reports from Europe (European Collaborative Study, 1988), France (Blanche *et al.*, 1989), Zaire (Ryder *et al.*, 1989), and Haiti (Halsey *et al.*, 1990) agree on a transmission rate of around 30 per cent, although the most recent European data suggests a vertical transmission rate of 13 per cent (European Collaborative Study, 1991). In the families under

study here, 10/53 (=19 per cent) or 8/47 (=17 per cent) of unrelated children acquired HIV from their mothers, but our sample was biased towards infected children. Data based on children over 18 months old in the Edinburgh cohort under prospective study indicate a mother to infant transmission of only three out of 47 or 6 per cent (Mok *et al.*, unpublished data). This rate is substantially lower than we expected and may be related to genetic characteristics of our study population.

The HLA antigen frequencies in the babies reported here, representing a relatively deprived social group, differed somewhat from our controls, which were babies of healthy parents unselected for HIV status but who were by comparison affluent and with no known history of intravenous drug use. Most notably, the relative incidence of the two commonest HLA antigen combinations (which usually occur as haplotypes) were reversed. Our data are consistent with an earlier report that A1, B8, DR3 may be weakly associated with susceptibility to infection (Steel *et al.*, 1988), and support the speculation that A3, B7, DR2 might confer some measure of protection.

On the basis of this small survey, it is clear that tissue typing would be of little value as a predictive test in babies born to mothers known to be HIV positive. There were no absolute differences in any antigenic specificity when comparing HIV-positive and HIV-negative neonates, and our family studies revealed no obvious relationship between haplotype inheritance and disease susceptibility or progression. However, HLA antigens may be associated directly or indirectly with susceptibility to transplacental infection and this might manifest itself as varying transmission rates in different populations. In particular, a high A3, B7, DR2/A1, B8, DR3 ratio may contribute to a low transmission rate, and this possibility may merit further investigation as a specific hypothesis.

Our results must be considered preliminary, but this is the first investigation into genetic susceptibility to HIV infection in infants of seropositive mothers. This study therefore could form a useful starting point for larger and more detailed surveys.

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Use of Intravenous Immunoglobulin in Acquired Immune Deficiency Syndrome

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Patients infected with the human immunodeficiency virus (HIV) may have an antibody deficiency and a deficiency of cellular immunity. Intravenous immunoglobulin (IVIG) preparations may benefit HIV-infected children and adults with recurrent bacterial infections at doses of 200 to 400 mg/kg every 2 to 4 weeks. In addition, IVIG (1 to 2 g/kg) is effective at raising platelet counts to hemostatic levels in HIV-infected patients with idiopathic thrombocytopenic purpura and life-threatening bleeding. Indirect evidence also suggests that IVIG may be effective in preventing *Pneumocystis carinii* pneumonia. Finally, recent studies suggest that specific anti-HIV antibody preparations may have a therapeutic role, either as immunoglobulin concentrates or as immunoadhesins and immunotoxins. However, further investigations are needed to exclude antibody enhancement of HIV infection by the Fc receptor or the complement receptor. *Cancer* 68:1440–1450, 1991.

PATIENTS with acquired immune deficiency syndrome (AIDS) and other symptoms associated with human immunodeficiency virus (HIV) infection have a severe deficit of cellular immunity associated with the progressive depletion of CD4-positive helper-inducer lymphocytes. As a result, they are infected with various opportunistic organisms, and attempts at immunotherapy primarily focused on treating the cellular immunodeficiency. However, patients infected with HIV also have other immunologic deficits, including various abnormalities of B-lymphocyte function (Table 1). Some of these abnormalities may be a result of the lack of adequate CD4 helper cell function associated with the decline in the CD4 helper cell numbers that occurs with progression of HIV disease. Despite the hypergammaglobulinemia observed, more frequent infections of the respiratory tract occur in children[1,2] and adults[3] with HIV infection, and such fections may be caused by encapsulated gram-positive ganisms. Because this pattern of infection is suggestive antibody deficiency, intravenous immunoglobulin (IVI therapy has been assessed, and early studies in ch dren[4–6] suggest that such therapy might be beneficial reducing the number of recurrent infections. Several ot studies support a role for IVIG in HIV-infected patie (Table 2), and this review will describe such studies HIV-infected children and adults, in HIV-infected patie with idiopathic thrombocytopenic purpura (ITP), a possibly in *Pneumocystis carinii* pneumonia (PCP). It also highlight some new developments that have occur since this subject was reviewed last.[7]

Intravenous Immunoglobulin for Children With Hum Immunodeficiency Virus Infections

Vertically transmitted infection with HIV in infa may result in a deficiency of antibody and cellular i munity. The usual clinical presentation in infants w recurrent bacterial infections is similar to that of X-link agammaglobulinemia. Despite the polyclonal increase serum immunoglobulin (Ig) concentrations, the poor p mary and secondary specific responses to antigen ch lenge render such infants susceptible to bacterial infecti Clinical benefit may therefore result from the admi

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Five had recurrent purulent pulmonary infections, and the sixth had recurrent prostatitis and mastoiditis. They were given infusions of 200 mg/kg IVIG (manufactured by the Scottish National Blood Transfusion Service) every 3 weeks as outpatients in an open study.[23] At each hospital attendance, both before and during IVIG treatment, symptoms of infection and antibiotic consumption were documented. At the end of the treatment period, the hospital records were scrutinized, and the antibiotic consumption and number of inpatient days because of infection were noted. For comparison, the same information was obtained from the hospital records for the 6-month period immediately preceding the start of IVIG therapy. Information concerning the dose of antibiotics prescribed was not analyzed, no distinction was made between intravenous and oral routes of antibiotic administration, and full compliance with prescribed oral antibiotic therapy was assumed.

A statistical analysis of the differences between the pretreatment and treatment periods in the percentage of time spent as an inpatient and the percentage of time receiving antibiotics was performed using the Wilcoxon rank-sum method on paired data. We found a statistically significant reduction in inpatient hospitalization ($P < 0.05$) and antibiotic consumption ($P < 0.05$) in the period after IVIG therapy compared with the preceding 6-month period when each patient was used as their own control (Fig. 3). Patients varied in the improvement associated with IVIG treatment. Although regular IVIG infusions did not prevent the occurrence of infections completely during the period of follow-up, there were fewer infections observed by a reduction in antibiotic requirements in all except Patient 4, whose requirement did not change. There was also a reduction in the hospitalization required for infection in all except Patients 4 and 6 (not shown), who did not spend any time as inpatients during control or treatment periods.

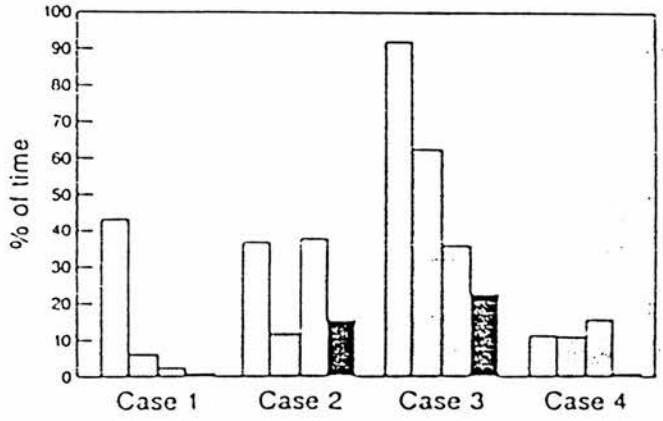

FIG. 3. Individual data for Patients 1 through 4 comparing percentage of time taking antibiotics before (stippled) and after (striped top left to lower right) IVIG therapy, and percentage of time in hospital before (striped top right to lower left) and after (solid) IVIG therapy.

The regimen of IVIG therapy administered was t same as that initially given as replacement therapy f primary hypogammaglobulinemia. Using 300 mg/ IVIG every 2 weeks, a similar improvement was seen one patient studied by others.[14] There is no consens concerning the optimal dose of IVIG or the frequency administration in this situation as is the case in the ma agement of primary hypogammaglobulinemia.

We were particularly interested in calculating the co of IVIG therapy because it is high (currently £15 (sterling g in the United Kingdom). We made the assumption th each patient would require inpatient hospitalization f the same percentage of time after the commencement IVIG as before its institution. The cost of materials w calculated on the basis of £15/g for the IVIG and £5 f the disposables used at each infusion, multiplied by t number of infusions administered. Staff time and oth factors required for each 2-hour infusion cost £87,[24] a inpatient care cost £200 per day.[25]

The cost of IVIG therapy (100 infusions) was £21,1 for the 1.41 kg of IVIG given, £500 for disposables, a £8700 for the staff and other costs, a total cost of £30,35 Treatment was associated with a reduction in hospit ization (Fig. 3), the difference between expected and o served hospitalization being 115.3 days for the six cas This resulted in an avoided expense of £23,060. Howev in this study, we chose to treat two patients with recurre infections who had not required hospitalization in t pretreatment period (Patients 4 and 6) and, therefore, f whom potential saving on hospitalization cost was no existent. Subtracting the cost of their treatment (27 i fusions of 360 g of IVIG, £7884) and considering the oth four cases only, the treatment costs were £22,466 co pared with an avoided expense for reduced hospitalizati of £23,060, suggesting that in carefully selected cases quiring hospitalization for infections, IVIG therapy m be cost-effective.

In summary, using an IVIG dose of 200 mg/kg eve 3 weeks, we showed in the four cases requiring hospit ization for recurrent infections that IVIG treatment w cost-effective. However, our preliminary data suggest that IVIG therapy may not be cost-effective in patien who do not require hospitalization for recurrent infectio and further studies, preferably of placebo-controlle crossover design, are required to determine the place this therapy in the management of the HIV-infected p tient with recurrent bacterial infections.

Intravenous Immunoglobulin in the Prevention and Treatment of Pneumocystis Infection

The most significant opportunistic infection in patien with AIDS, *P. carinii* is a major cause of morbidity a mortality, even in patients receiving treatment for H infection with zidovudine. Currently, the available tre

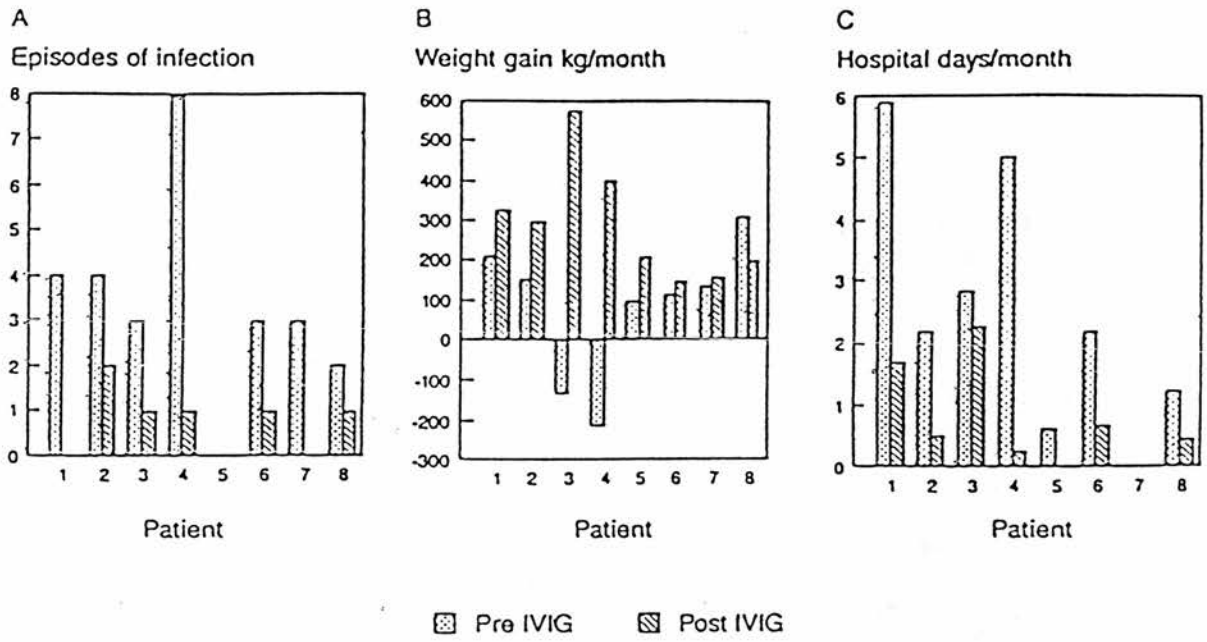

FIG. 1. Individual data showing reduction in (A) the number of episodes of infection, (B) weight gain, and (C) the number of days spent in the hospital in eight HIV-infected children treated with IVIG. Patient 5 had no infections before or after IVIG and Patients 1 and 7 had no infection after IVIG therapy. Patient 7 had no hospitalization before or after IVIG therapy and Patient 5 had no hospitalization after IVIG therapy.

but in none of the six patients with elevated concentrations at entry to the study did the levels normalize with treatment. On the basis of their results, the authors recommended that IVIG should become standard therapy in symptomatic pediatric HIV infections. However, in this pilot study, IVIG therapy was only of 6 months' duration in four of the seven children studied, and there was no adequate control period of comparison before the initiation of regular IVIG therapy.

Our group therefore undertook a prospective open study in which eight children with symptoms of HIV infection were treated for 12 to 26 months (median, 14 months) with three weekly infusions of IVIG (200 mg/kg).[10] Because of the heterogeneity of clinical symptoms in the HIV-infected children, particularly in the frequency of infection and weight gain, each child acted as their own control. A significant improvement was seen in all children during the IVIG treatment period in the number of infectious episodes, weight gain, and days spent in the hospital compared with the control period preceding IVIG therapy (Figs. 1A to 1C). The IVIG treatment resulted in a 49% saving in management costs compared with those arising previously as a result of inpatient admissions. Serum Ig levels, increased at the start of treatment, were not altered, and T4 counts continued to decline slowly (Fig. 2), as reported previously.[9]

One finding of particular interest was the observation that HIV core antigen, detected in four children before therapy, disappeared after IVIG therapy was commenced. This effect was sustained in three children.[10] We therefore postulated that IVIG therapy might reduce viral replication by preventing the lymphocyte activation and proliferation induced by bacterial infection and hence might delay the progression of HIV-related disease.

A critical issue in the therapy of HIV-infected children with IVIG is the point in the illness at which IVIG treatment should be initiated. We consider that after two or more serious infections, particularly with encapsulated gram-positive organisms, long-term treatment with IVIG is indicated. In the case of asymptomatic HIV-infected children and symptomatic HIV-infected children who do not have bacterial infections, *e.g.*, those with failure to thrive and lymphadenopathy, there may be a strong case

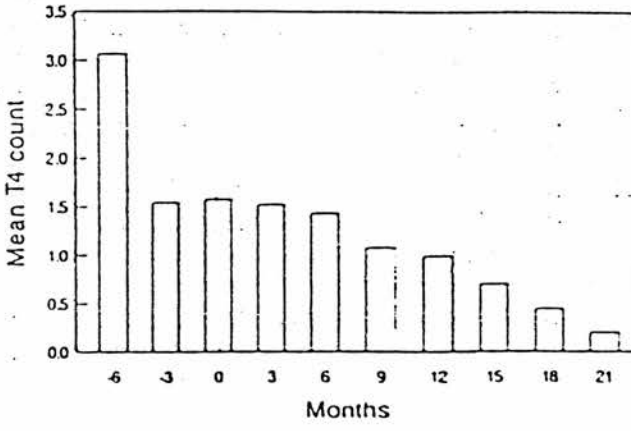

FIG. 2. Mean CD4 (helper) lymphocyte counts in eight HIV-infected children before and after IVIG therapy.

TABLE 1. Immunologic Abnormalities in Human Immunodeficiency Virus Infection

Quantitative abnormalities of T-lymphocytes
Decreased numbers of helper–inducer (CD4) cells
Elevated, normal, or decreased number of suppressor-cytotoxic (CD8) cells
unctional abnormalities of T-lymphocytes *in vivo*
Host susceptibility of infection
Host susceptibility of neoplasms
Decreased delayed-type hypersensitivity responses
unctional abnormalities of T-lymphocytes *in vitro*
Elevated spontaneous proliferation
Decreased proliferative responses to mitogens and antigens
Decreased virus-specific cytotoxic lymphocyte function
Decreased ability to provide help to B-lymphocytes
unctional abnormalities of B-lymphocytes *in vivo*
Elevated serum immunoglobulin levels
Circulating immune complexes
Inability to mount an appropriate serologic response after immunization
unctional abnormalities of B-lymphocytes *in vitro*
Elevated spontaneous proliferation
Elevated numbers of spontaneous plaque-forming cells in the peripheral blood
Enhanced responsiveness to B-cell growth factors
Refractoriness to the normal *in vitro* signals for B-cell activation
bnormal suppressor phenomena in AIDS
Suppressor factors in sera
Antilymphocyte antibodies
T-cell–derived suppressor substances

AIDS: acquired immune deficiency syndrome.
Reproduced with permission from Lane HC, Fauci AS. Immunologic bnormalities in the acquired immunodeficiency syndrome. *Annu Rev mmunol* 1985; 3:477–500.

ration of IVIG due to its content of antibodies with a ide range of specificities, including those against gram-ositive encapsulated bacteria. Several studies have at-empted to evaluate whether infants and children with IDS or AIDS-related complex (ARC) benefit from IVIG herapy.[4–10]

In one retrospectively reviewed series of 31 children ith AIDS treated at the Children's Hospital of New Jersey etween 1981 and 1983, 19 were treated with 200 mg/kg VIG every 4 weeks for at least 5 months, and 12 received o IVIG therapy. The two groups were comparable with regard to symptoms and clinical course, and over a period of follow-up of at least 2 years for each patient, only three of the IVIG-treated children died (16%) compared with ten of the control group (83%), a significant difference in mortality between the two groups occurring in association with IVIG therapy.[8]

In another study in New York, 14 of 41 children with AIDS and recurrent bacterial infections were treated with a course of IVIG consisting of infusions of 50 and 100 mg/kg during week 1, 150 and 200 mg/kg during week 2, 200 mg/kg twice during week 3, and 300 mg/kg during weeks 4, 6, and 8.[5] The remaining 27 patients did not receive IVIG therapy, although the basis for selecting which cases to treat with IVIG was not clear. Over a period of follow-up of 20.4 ± 9.2 months, only three febrile episodes lasting longer than 1 week occurred in the 14 cases treated with IVIG, with one episode of sepsis. In the 27 untreated cases, there were 26 such febrile episodes during the same follow-up period, with 18 episodes of sepsis. Given the historically controlled or uncontrolled nature of these studies, it is difficult to be certain of the efficacy of IVIG therapy in reducing either the morbidity from bacterial infections or the mortality in children who have AIDS.[5]

A more detailed analysis of the clinical benefit of IVIG therapy in HIV-infected children was undertaken by others.[9] In a prospective study of seven HIV-infected children for 6 to 24 months after the start of IVIG therapy, the treatment schedule consisted of IVIG at a dose of 0.4 g/kg on 5 consecutive days and then 0.4 g/kg every 2 weeks for 3 months, followed by 0.4 g/kg every 4 weeks. There was a sharp reduction of febrile and infectious episodes, a normalization of physical and psychomotor development, and an absence of mortality. However, no beneficial effect of IVIG on cellular immunity in terms of *in vitro* lymphocyte responses to mitogens and skin test reactivity to recall antigens was observed, and a gradual decrease in total and CD4-positive helper lymphocytes occurred during the course of the study. The mean value of circulating immune complexes was lower during treatment.

TABLE 2. Some Applications of Intravenous Immunoglobulin Preparations in Human Immunodeficiency Virus-Infected Patients

Patient group	Dose	Comment
hildren with recurrent bacterial infections	200–400 mg/kg every 2–4 weeks	Value of IVIG therapy in asymptomatic HIV-infected children unknown
dults with or without recurrent bacterial infections	200–400 mg/kg every 2–4 weeks	Trials in progress—value of this therapy unknown
TP associated with HIV infection	1–2 g/kg over 1–5 days	Therapy with zidovudine may be an alternative; useful for life-threatening bleeding
se to delay progression to AIDS	Not known	Specific anti-HIV antibody preparations required; initial trials in progress

ITP: idiopathic thrombocytopenic purpura; HIV: human immunodeficiency virus; AIDS: acquired immune deficiency syndrome; IVIG: intravenous immunoglobulin.

starting IVIG. It is possible that intercurrent infection
n organisms other than HIV may be a co-factor in the
gression of HIV infection.[7]
inally, there remains the question of whether all in-
s born to HIV-seropositive mothers should be con-
red for IVIG therapy. The main difficulty lies in the
onstration in infants of HIV infection: all infants born
eropositive mothers have circulating anti-HIV anti-
y of maternal origin that disappear after a variable
od. Furthermore, the current low vertical transmission
s (15% to 20%) make it unlikely that IVIG will be
pted generally in the management of HIV-infected
dren before the development of symptoms. Resolution
is issue will depend on better laboratory methods for
diagnosis of HIV infection in the first 6 months of
such as the use of the polymerase chain reaction.[11]
summary, two early retrospective studies and two
nt prospective studies, all with relatively small num-
of symptomatic HIV-infected children, indicated that
tment with IVIG at doses of 200 to 400 mg/kg every
4 weeks is associated with clinical benefit. However,
inal proof of the efficacy of IVIG will await the results
he large, double-blind, placebo-controlled trial in
ptomatic HIV-infected children now in progress in
United States. To date, no studies of IVIG have taken
e in asymptomatic HIV-infected children, and such
ies will have to await the general availability of meth-
of diagnosing HIV infection early in life.

ravenous Immunoglobulin for Adults With Human Immunodeficiency Virus Infection

dults with AIDS have profoundly impaired cellular
unity and suffer various opportunistic infections.
e patients infected with HIV, especially intravenous
abusers, whether or not they have progressed to
S, have more frequent bacterial infections of the re-
tory tract, suggesting specific antibody deficiencies.[3,12]
organisms usually implicated are encapsulated gram-
ive bacteria, and the overall clinical picture bears a
g resemblance to primary antibody deficiency syn-
nes. Thus, it is possible that replacement therapy with
G may be beneficial in such patients. However, studies
uating the efficacy of IVIG therapy in HIV-infected
t patients are difficult to do because only a minority
recurrent bacterial infections, they are heterogeneous
regard to symptoms and laboratory indices, and it
ficult to control for the effects of other factors such
garette smoking and continued drug abuse. Although
tudy showed a decrease in mortality in HIV-infected
s treated with IVIG,[13] there were no significant re-
ons in the number of bacterial or viral infections.
e are currently no published reports of any trials of
treatment in HIV-infected adult patients with re-
current bacterial infections and only one anecdotal report about a single HIV-infected adult with recurrent pyogenic infections who appeared to benefit significantly from IVIG therapy.[14]

We can make a strong case for reducing intercurrent infections in HIV-infected adult patients because it is possible that infection with organisms other than HIV may be a co-factor in the progression of HIV-related disease. Respiratory infections are common in HIV-infected patients,[15] but accurate estimates of the proportion of HIV-infected patients with recurrent bacterial infections currently are not available.

Infections should occur more frequently when specific antibody secretion is impaired, and cases with such impairment should benefit from IVIG therapy. Lower levels of antipneumococcal antibody are found in homosexual patients with AIDS than in seronegative heterosexual control subjects: a poorer antibody response occurs after vaccination with pneumococcal vaccine.[16] In addition HIV-seropositive homosexual patients with ARC have lower postimmunization titers than heterosexual control subjects,[17] and a defective response to immunization was found in seropositive hemophiliac patients.[18] If the ability to produce an antipneumococcal antibody response were regarded as a marker for the ability to produce antibodies directed against other gram-positive organisms, then one logical approach would be to commence IVIG in cases where the serologic response to successive pneumococcal vaccinations declined.

In an attempt to assess whether such therapy is as beneficial in adults as it is in children, we recently undertook a pilot study in a group of six HIV-infected adult patients with recurrent bacterial infections, the results of which were reported briefly elsewhere.[19] We chose to select only patients with recurrent bacterial infection and to study each patient as their own control. A comparison of consecutive periods with and without IVIG therapy was used; each patient should have remained unchanged clinically or become worse (as a result of the progressive nature of HIV infection) rather than better during the period of IVIG therapy. No change in other prescribed drug therapy (such as zidovudine) was made during the study, and there were no changes in the patterns of continuing drug abuse in current abusers (data not shown).

To evaluate the efficacy of IVIG therapy, we used two relatively simple indices—days in the hospital and days of antibiotic use. These were useful in the evaluation of this therapy in patients with primary hypogammaglobulinemia[20-22] and HIV infection.[10] Each patient studied had recurrent infections that did not respond satisfactorily to antibiotic treatment before IVIG therapy. The cases studied were six adults whose HIV infections were acquired by blood transfusion (Patient 1), intravenous drug abuse (Patients 2 to 5), and homosexuality (Patient 6).

TABLE 3. Effect of Intravenous Immunoglobulin in Human Immunodeficiency Virus-Associated Idiopathic Thrombocytopenic Purpura

Author (year)	No. of patients treated	IVIG dose	Patients responding*	Range of peak platelet counts (10^9/l)
Panzer *et al.* (1985)[40]	5	1 g/kg (2) 2 g/kg (3)	5	78–250
Imbach *et al.* (1987)[41]	4	1.6 g/kg (2) 2 g/kg (2)	4	> 150
Tertian *et al.* (1987)[43]	11	1 g/kg (8) 2 g/kg (3)	9†	58–193
Oksenhandler *et al.* (1987)[42]	17	1.2–2.0 g/kg	12	
Bussel and Haimi (1988)[45]	22	1–2 g/kg	19	86–404
Pollack *et al.* (1988)[44]	3	2 g/kg	3	60–215
Beard and Savidge (1988)[45]	5	2 g/kg	5	97–300
Landonio *et al.* (1990)[32]	17	2 g/kg	5	Not stated
Total	84		62 (75%)	

IVIG: intravenous immunoglobulin.
* Initial increment in platelet count to > 50 × 10^9/l.
† Six responders received 1 g/kg of IVIG.

two recent reports showed that a dose of 1 g/kg over 2 days was equally effective in raising the platelet count in both acute and chronic ITP patients (Table 3).[43,44] We had similar results in the treatment of both HIV-infected cases and classic ITP with six of our first 12 patients achieving a useful rise in platelet count by day 3 (platelet count, > 30 × 10^9/l; A.A.M.T., P.E.W., and P.L.Y., unpublished data). In addition, when maintenance therapy is required, this can be reduced from the standard 1 g/kg to 0.5 g/kg without apparent detriment in terms of initial response or duration of response.[45] In children, the cost of a course of IVIG therapy is relatively inexpensive and may be recouped by the reduced need for hospitalization.

The mechanism of action of IVIG is not understood completely.[46] Modulation of Fc receptor expression and Fc receptor blockade were found, and there is increasing evidence that reticuloendothelial blockade is the main mechanism of action, at least in the immediate response. Inhibition of antibody synthesis may occur by a direct effect on B cells or modulation of T-suppressor activity. Antiidiotype antibodies in the IVIG preparation could suppress aberrant innate idiotype–antiidiotype control mechanisms that allow the production of antiplatelet antibodies. Alternatively, the IVIG may act by clearing a putative viral agent (although evidence for this is lacking).

Others[47] recently found increased production of the cytokines, tumor necrosis factor (TNF) and interleukin-1, in HIV-infected monocytoid cells. These substances have a potentially suppressive effect on hemopoiesis, and an additional hypothesis for an effect of IVIG on platelet production can be postulated. Suppression of TNF production by rabbit macrophages was found using a human IgG preparation.[48] This mechanism has not been investigated in HIV-associated ITP to date.

Anti-Rh (anti-D) Ig therapy was valuable in patients with classic ITP and, more recently, in HIV-associated cases.[49,50] The effect appears to depend on an interaction between the Rh antibody and its antigen because anti-D is only effective in D-positive patients unless given as *in vitro*-sensitized cells. The therapy may divert the reticuloendothelial system from platelet destruction to sequestration of IgG-coated erythrocytes. The efficacy appears similar to that of high-dose IVIG, but when used at the doses reported, it can be less expensive. Transient positivity of the direct Coombs test and rise in bilirubin are seen, but significant hemolysis is not normally a problem. The limited availability of anti-D suitable for intravenous administration currently inhibits more widespread use of this treatment, but this may no longer apply when monoclonal anti-D preparations become available.

Finally, the requirement for specific therapy for ITP may diminish with the more widespread use of zidovudine.[51] There is increasing evidence showing the efficacy of zidovudine in raising the platelet count in approximately 50% of those treated, at least in part by stimulating increased marrow production.[52] This lends support to the hypothesis that HIV may have a direct role in the pathogenesis of the ITP.

In summary, IVIG appears to have a useful role in the treatment of HIV-related ITP, having at least a transient effect in most of those treated and lacking the immunosuppression that is a potential disadvantage of many alternative therapies. The main disadvantage of its cost may be reduced by using a reduced dose schedule that appears to be equally effective. The possibility of unlimited supplies of monoclonal anti-D offers exciting prospects for an economic alternative therapy. Zidovudine may negate the requirement for other treatments in a large proportion of HIV-infected thrombocytopenic patients.

Therapy Based on Specific Anti-Human Immunodeficiency Virus Antibody Preparations

Only IVIG preparations derived from healthy blood donors have been considered thus far. These preparations

not contain any anti-HIV antibodies, unlike specific -HIV Ig preparations. When used for the prevention HIV infection, specific anti-HIV preparations might in several ways (by neutralizing free virus in the blood, venting binding to the target cell, or preventing early y into the cell). Such preparations also may play a in preventing the progression of HIV-related disease increasing the interval from primary HIV infection ne development of AIDS. They may be useful in combation with other therapies, *e.g.*, with continuous antotics, antiviral agents, such as zidovudine, and with G.

major difficulty with developing an anti-HIV Ig paration is in defining the mechanism by which HIV ts its cytopathic effect and the fine specificity of the -HIV antibodies required. Several events occur during cycle of HIV infection and replication, which could to cell death.[53] In some of these steps, specific anti- antibody preparations may have a therapeutic role.[7] vever, a major difficulty in their development lies in ning which antibodies, if any, confer protection *in vivo*. re are various reports of the existence of neutralizing bodies in the serum of HIV-infected patients and also ntibody-dependent cellular cytotoxicity (ADCC), ining antibodies against HIV. It appears that ADCC bodies may be related to neutralizing antibodies.[54] An ciation between virus neutralizing activity and the cal progression of HIV disease was found,[55,56] sug- ng that neutralizing antibodies can be protective fac- during the evolution of HIV-related disease. However, rent isolates of HIV strains showed a variability in xtent of sensitivity to neutralization by sera obtained different HIV-infected individuals,[56] and this vari- ty has been attributed to the existence of various HIV ypes.[57] The mechanism for antibody neutralization investigated further by the use of monoclonal anti- es,[7] and relatively specific neutralization epitopes were gnized.[58] One study indicated that there are at least classes of biologically active antibodies to HIV.[59] One of antibodies is isolate restricted and directed pri- ly to a hypervariable loop structure of gp120 that is nvolved in CD4 binding. The second class of neu- zing antibodies is directed at a more conserved struc- which may include those involved in CD4 binding. addition to preventing binding of HIV to lympho- or monocytes, anti-HIV antibodies may prevent age caused by toxic components of HIV. For instance, native and recombinant gp120 proteins from HIV- re found to produce neuronal injury in the mam- an central nervous system, and this neurotoxicity was ght to involve a rise in intracellular Ca^{++}. Immu- ecipitation of gp120, using anti-gp120, could prevent rise,[60] and this observation may be relevant for the unopathology of HIV outside the central nervous m. Another possible application of specific anti-HIV antibody preparations may be in preventing primary infection after needle-stick injuries: zidovudine prophylaxis will not necessarily prevent HIV-1 infection.[61] The use of antibody preparations for this application would be similar to the use of specific Ig preparations for the postexposure prophylaxis of hepatitis B, rabies, measles, and varicella-zoster. However, it should be noticed that there is a very high affinity of gp120-CD4 interaction with an affinity constant of approximately 4×10^{-9}.[62] An antibody effectively inhibiting this should have an affinity tenfold to 100-fold higher (10^{-10} or 10^{-11}), and thus it may be difficult to produce. Large doses of antibody might have to be administered for postexposure prophylaxis, and virus also might be transmitted by cell-to-cell contact: antibodies might not gain access to relevant epitopes.

The production of specific anti-HIV Ig preparations may be difficult, but two studies were done, using anti-HIV antibodies in the form of plasma. There have been three attempts made to produce specific anti-HIV Ig preparations. In one study,[63] the infusion of plasma rich in anti-p24 antibodies, collected from healthy HIV-infected persons, resulted in the clearance of HIV antigenemia. These plasma infusions were followed by fewer symptoms of HIV disease, a reduction in the frequency of opportunistic infections, and a decline in the rate at which HIV could be cultured from plasma or lymphocytes. Another similar study[64] used plasma rich in neutralizing antibodies. However, the results from this study are difficult to interpret, and the results of further studies using plasma containing high levels of anti-HIV antibodies are not known currently.

Three groups attempted to prepare specific anti-HIV Ig preparations. One group from New York used such a preparation, manufactured from plasma rich in neutralizing antibodies against HIV, in protection experiments involving chimpanzees.[65] Unfortunately, all four chimpanzees in the experiment became infected with the same strain of HIV used in the neutralization assays *in vitro*. The period of incubation to virus isolation in specific anti-HIV Ig recipients did not differ significantly from the corresponding period observed in a control chimpanzee that received normal anti-HIV-free Ig. In a detailed analysis of this disappointing result,[66] the authors suggested several reasons for the experimental failure, including the possibility that too high a challenge dose of HIV was used, although Fc receptor-mediated, antibody-dependent enhancement, or complement-mediated, antibody-dependent enhancement also may have occurred. Results from repeat experiments using a lower dose of HIV are anticipated.

Another anti-HIV Ig preparation, manufactured in Japan, was shown to prevent the spread of HIV *in vitro*.[67] However, no *in vivo* experiments using this globulin preparation are available yet. Finally, another group from the United States also developed an anti-HIV Ig preparation.

and initial clinical trials showed that the half-life of the anti-p24 antibody administered was 16 days. In Ig recipients who were HIV p24 antigen positive, the half-life was shorter.[68] Large-scale clinical trials of such specific anti-HIV immune globulin preparations may be difficult to perform because of the limited availability of suitable plasma for fractionation. Although HIV-antibody-positive blood donors could be used as a source of neutralizing antibody, the screening of such donors, the collection of their plasma, and its fractionation to yield a noninfective IVIG preparation would present formidable challenges. Furthermore, removal of plasma containing neutralizing antibodies from HIV-antibody-positive donors might cause them harm. Mouse and human monoclonal neutralizing antibody cell lines have been developed,[7] but the former may only be suitable for single courses of therapy, and production of the latter is limited by the supply of immune B-cells.

Finally, Ig molecules may be involved in two alternative approaches to the therapy of HIV infection. First, there is increasing interest in the use of soluble CD4 molecules as a means of diverting free HIV virions from infecting susceptible host cells. However, soluble CD4 has a short half-life, necessitating frequent injections, and a chimeric molecule was developed where the Fab region of the IgG molecule is replaced by CD4.[69] Such chimeric molecules, or CD4 immunoadhesins, were shown in primate experiments to possess the gp120 binding and HIV blocking properties of recombinant soluble CD4 and the long plasma half-life of IgG, Fc receptor binding, the ability to mediate ADCC, and to cross the placenta.[70] A pentameric CD4-IgM chimeric molecule, which is at least 1000-fold more active in syncytium inhibition assays, with retention of Fc binding and the ability to activate complement, also was described.[71]

Second, the use of antibody–toxin (immunotoxin) conjugates in HIV infection was investigated *in vitro*.[72] Such a conjugate using monoclonal anti-gp120 with ricin A chain was shown to cause a dramatic reduction in the number of infectious virions produced by immunotoxin-treated cells. It will be interesting to see how such an immunotoxin compares with the use of a recombinant CD4-Pseudomonas exotoxin hybrid protein which has recently been reported to kill HIV-infected cells selectively.[73]

Conclusions

In general, IVIG preparations are safe in HIV-infected patients, and there is only one published report of a serious adverse reaction, involving an HIV-infected child where rapid administration of IVIG caused neurologic symptoms suggestive of the hyperviscosity syndrome.[74] However, there has been considerable interest in the possibility that antibody administration may enhance HIV infection[75] by increasing the entry of HIV into the cell through the Fc receptor[76,77] or the complement receptor.[78] More recently, serum enhancement of HIV infection was correlated with the clinical stage of disease in HIV-infected individuals, suggesting that enhancing antibodies may contribute to the progression of HIV disease.[79] Although more *in vivo* data on the importance of anti-HIV antibodies enhancing HIV infection are required before the significance of these observations is understood fully, the possibility that specific anti-HIV antibody preparations are harmful must be excluded before the widespread introduction of specific anti-HIV Ig preparations. Nonetheless, IVIG preparations have an important role to play in the management of HIV-infected patients and should be used more widely in the prevention of recurrent bacterial infections, and in the treatment of ITP.[80–85]

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Prognostic Value of HIV Antigen and Core Antibody Measurement in HIV-Infected Children: A Longitudinal Study

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o evaluate the prognostic significance of
nunodeficiency virus antigen (HIV Ag)
tibody (p24 Ab) levels in children, blood
re collected every three months from 11
infected children, aged 0–47 months
months) over a period of 4–57 months
months). The five children who had HIV
ml and were negative for p24 Ab showed
ly more rapid decline in $CD4^+$ lympho-
and were more likely to progress clini-
those with lower or negative HIV Ag
of 6 patients treated with intravenous
ulin, a sustained reduction in HIV Ag
bserved, but failed to reach significance,
cause of the small numbers of patients
effect could be seen on p24 Ab levels.
d change in HIV Ag or p24 Ab levels was
llowing zidovudine therapy. Plasma rich
as infused in 2 children, and was associ-
he elimination of HIV Ag from the
s effect was present only during the pe-
sma therapy, however, and no clinical
d be demonstrated. As HIV antigenemia
4 children before symptoms were appar-
spective follow up of all children born to
d mothers is important, so that those
arkers suggestive of poor prognosis can
for therapeutic intervention.

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Introduction

Human immunodeficiency virus (HIV) core protein, or p24 antigen (HIV Ag), can be detected in the plasma of HIV-infected individuals.[1] Studies in adults have shown that in primary infection, detection of HIV Ag may precede detection of HIV antibody.[2] Subsequent disappearance of HIV Ag may be due to a low level of virus replication or to the formation of immune complexes after the initial antibody response. Reappearance of detectable HIV Ag, together with a decline in HIV anticore antibody (p24 Ab) levels, is associated with progression of clinical HIV disease.[3] A larger proportion of asymptomatic children than adults have persistent antigenemia.[4] While this might reflect a different balance between active and latent HIV infection in children,[1] persistent antigenemia has been reported to be associated with disease progression and a poor prognosis.[5] No reports are available on the course of HIV Ag and p24 Ab levels in children over periods longer than 2 years, for correlation with the progression and clinical symptomatology of HIV infection. Our aim in this study of 11 HIV-infected children was to measure HIV Ag and p24 Ab levels quantitatively, and relate the measurements to the onset of symptoms, clinical progression, and therapeutic intervention.

Patients and Methods

Eleven children born to HIV seropositive mothers with clinical and laboratory evidence of HIV infection were identified at age 0–47 months (median 21 months). Three had been followed since birth, two were identified while still asymptomatic, and 6 presented with symptomatic disease. The median duration of follow up was 21 months (range 4–57). In

urviving, the median follow up was 36 (range 12–57).

Ag was measured by quantified enzyme im-say (Abbott, UK), and expressed as pg/ml, e standard supplied by the manufacturer with cut-off of 3 pg/ml. P24 Ab was also mea-ing the Abbott HIV-1 anticore enzyme im-ay. A quantitative value, expressed as p24 x, was derived by dividing the optical den-e of the sample tested by the cut-off value, tracting this from 1.0 (1-OD sample/OD serum). Thus, the higher the value the he competition (and amount of p24 AB) in le. Zero values indicate lack of antibody.

children (cases 1–3, 5, 7–11) received intra-mmunoglobulin (IVIgG) infusions of 200 very 3 weeks.[6] Plasma was obtained by plas-sis from donor 11, selected during a previ-y by Jackson et al.[7] for high serum titers of and neutralizing antibody and treated as ly described.[7] Plasma was titered for anti-i p41/gp120 and neutralizing antibody, but not for antibody to the hypervariable loop. It was then infused in patients 1 and 5 at a dose of 2 ml/kg every 6 weeks initially, increasing to every 3 weeks after three infusions in case 5. Five children (cases 1,2,5,7,8) received oral zidovudine at doses ranging from 400–600 mg/m^2/day.

Results

Of the 11 children, 10 had symptoms and signs of HIV infection during the study period. One child (case 6) had lymphadenopathy, hypergammaglobulinemia (IgG of 20 g/L) and a reversed T4/T8 ratio (0.8), but remained clinically well. Clinical details of all 11 children are summarized in Table 1, and laboratory data in Table 2. Of the five children (cases 1–5) who were negative for core antibody, and had HIV Ag levels above 50 pg/ml, four have died at ages 67 months (case 1), 43 months (case 2), 25 months (case 3), and 4 months (case 4), and one (case 5) has progressed to AIDS at age 51 months (group 1). Five children (cases 7–11) had low or

Table 1. Clinical Data on the HIV-Infected Children (Numbers = Age in Months)

Last Seen	Length of Follow Up	1st Symptoms	Progression to P2B-D[a]	Current Stage[a]	IV IgG Started	AZT Started	Symptoms
67 (D)	21	9	45	Dead	47	49	Recurrent pneumonia Esophageal candida Failure to thrive (wasting)
43 (D)	21	22	26	Dead	26	28	Recurrent pneumonia Encephalopathy PCP
25 (D)	14	22	24	Dead	23	—	Oral candida Encephalopathy Wasting syndrome
4 (D)	4	1	4	Dead	—	—	Oral and cutaneous candida PCP
72	33	12	51	P2C/E	36	58	ITP LIP Ewing's sarcoma
33	12	—	—	P1B	—	—	Asymptomatic
57	57	3	—	P2A	9	28	Recurrent pneumonia (at <1 yr) Molluscum contagiosum
69	51	9	—	P2F	24	42	Recurrent pneumonia Molluscum contagiosum Cardiomyopathy
48	48	9	—	P2F	14	—	Recurrent respiratory infection ITP
63	27	33	—	P2A	39	—	Recurrent respiratory sepsis Diarrhea
63	36	18	24	P2C	24	—	Recurrent pneumonia

lassified by CDC.[13] *Abbreviations:* (D) = Age at death; ITP = immune thrombocytopenic purpura; PCP = *Pneumocystis* umonia; LIP = lymphocytic interstitial pneumonitis.

HIV Ag values, and of these none has died essed to AIDS during the period of follow 57 months (group 2). Two children (cases 5 mained positive for p24 Ab, but were also genemic (group 3). Both were clinically hat time; case 6 has remained stable, but in bsequent loss of p24 Ab preceded clinical ion. Group 1 children also showed a signif- reater rate of fall in $CD4^+$ cells than 2 children ($p < 0.01$, Wilcoxon rank . $CD4^+$ lymphocytes remained stable in

e available on 6 children in the first 2 years d 3 were followed from birth. Case 4 was or p24 Ab from birth, with rapidly increas- Ag levels to 145 pg/ml at her death at the months. Cases 6, 7, and 8 had positive p24 Ab initially, but levels declined, coin- h emergence of low levels of HIV Ag. In dren, who remained clinically stable, HIV e negative and p24 Ab was subsequently as illustrated by case 9 in Figure 1.

ildren received IV IgG for 2–48 months 4 months). Of these, 6 (cases 1,2,3,7,8,9) tive for HIV Ag when therapy was com- Case 3 received IV IgG for only 2 months death, but during this time HIV Ag levels from 62 to 0 pg/ml. Data on case 5 are e. Of the remaining 5 children, there was a reduction in HIV Ag in 4 (cases 2,7,8,9), sly reported,[6] but numbers are too small to stical significance. No effect of IV IgG on vels was observed.

and 5 received plasma rich in anti p24 on two and six occasions, respectively. received IV IgG infusions for 18 months. (case 1) had received zidovudine in the past, but this had been discontinued because of toxicity 3 months previously. She was extremely ill with unresolving pneumonia, and died 8 weeks later. Case 5 remained clinically stable during treatment. The plasma infusions were effective in clearing HIV Ag completely, and raising p24 Ab levels 2 hours after completion of the infusion (Fig. 2). However, as p24 Ab levels declined, HIV Ag reappeared between 7 and 14 days later, returning to the pretreatment level by 6 weeks. On the 3-weekly infusion regimen, HIV Ag reemerged despite positive p24 Ab testing.

Five children received zidovudine. No clear relationship was observed between commencement of therapy and change in HIV Ag or p24 Ab levels (Fig. 3). HIV Ag reemerged during therapy in cases 1, 5, and 8 after 1–14 months, probably representing zidovudine resistance.

Discussion

Our results indicate that HIV Ag assay is valuable as an early diagnostic test in children born to HIV seropositive mothers. Nine of eleven infected children were positive for HIV Ag, 6 of whom were at the time either asymptomatic or had symptoms not specific for HIV infection. Our study also emphasizes the importance of regular prospective follow up of all children born to HIV-infected mothers, because peak HIV Ag levels may occur in the asymptomatic phase and would be missed if the child were not identified prior to becoming ill.

One previous study has correlated persistent HIV antigenemia and loss of p24 Ab with a poor prognosis.[5] Our results suggest that high levels of HIV Ag in the absence of p24 Ab rather than the duration of antigenemia are significant in predicting those HIV-

Table 2. Laboratory Data on the HIV-Infected Children

1st Sample (Months)	1st Ag^+ (Months)	Peak Ag (pg/ml)	Last Ag (pg/ml)	1st Ab^- (Months)	Last Ab (Index)	Last T4 Count ($\times 10^9$/ml)	Rate of T4 Decline (Average/Year)
47	47	>150	>150	47	0	0.01	95%
24	24	97	2	24	0	0.01	88%
11	15	>150	0	11	0	0.02	99%
0	2	145	145	0	0	1.08	90%
42	42	>150	>150	49	0	0.10	60%
21	21	>150	48	—	0.42	1.11	0%
3	9	11	0	6	0.59	0.60	15%
18	24	28	15	24	0	0.68	25%
3	6	21	0	6	0.50	0.67	18%
36	—	0	0	—	0.77	0.60	25%
24	—	0	0	—	0.94	0.60	10%

children whose disease will progress rap-
ır observation of a difference in the rate of
of $CD4^+$ lymphocytes between groups 1 and
en described in adults,[8] but there has been no
report in children.
line in p24 Ab has been reported to precede
tigenemia as a correlate of prognosis in
In children born to HIV seropositive
such a finding is difficult to interpret be-
the inevitable decline of passive maternal
levels in the first year of life. There have
previous reports relating p24 Ab levels at
th progression of disease in children, al-
he maternal p24 Ab level during pregnancy
appear to correlate with the risk of transmis-
Our preliminary longitudinal study of three
suggests that absence of maternal p24 Ab at
y be associated with rapid progression of
ociated disease in children who are infected,
er studies on larger groups of children are

f the children studied (group 3) were simul-
y p24 Ab+ and HIV antigenemic. In a pre-
port,[4] this feature was found in half of the
studied, but no attempt was made to corre-
finding with prognosis. In adults it occurs infrequently.[8] This may be because high levels of antibody are produced as a response to HIV Ag by the immune system of the child. It is possible that active viral replication is more common in children; the rate of spontaneous mutation of HIV is such that the p24 Ab produced by the host lacks specificity, and thus avidity. It is interesting, however, that in the two children who were concurrently positive for antibody and antigen this state was not associated with rapid clinical deterioration.

We have previously reported a trend toward reduced HIV Ag levels following commencement of IV IgG therapy,[6] and we have found that this effect persists for 3–48 months. However, in the current study reduction in HIV Ag levels was not sufficiently marked to reach statistical significance in the small number of children studied. One possible mechanism for HIV Ag suppression is that bacterial and viral infections can cause lymphocyte activation, and trigger HIV gene expression. The reduction in the number of episodes of infection achieved by IV IgG may therefore reduce HIV activation.

The use of plasma rich in p24 Ab has not been reported in children, although clinical benefit has been described in adults.[7] We therefore chose a dose of plasma equivalent to that which has been used in

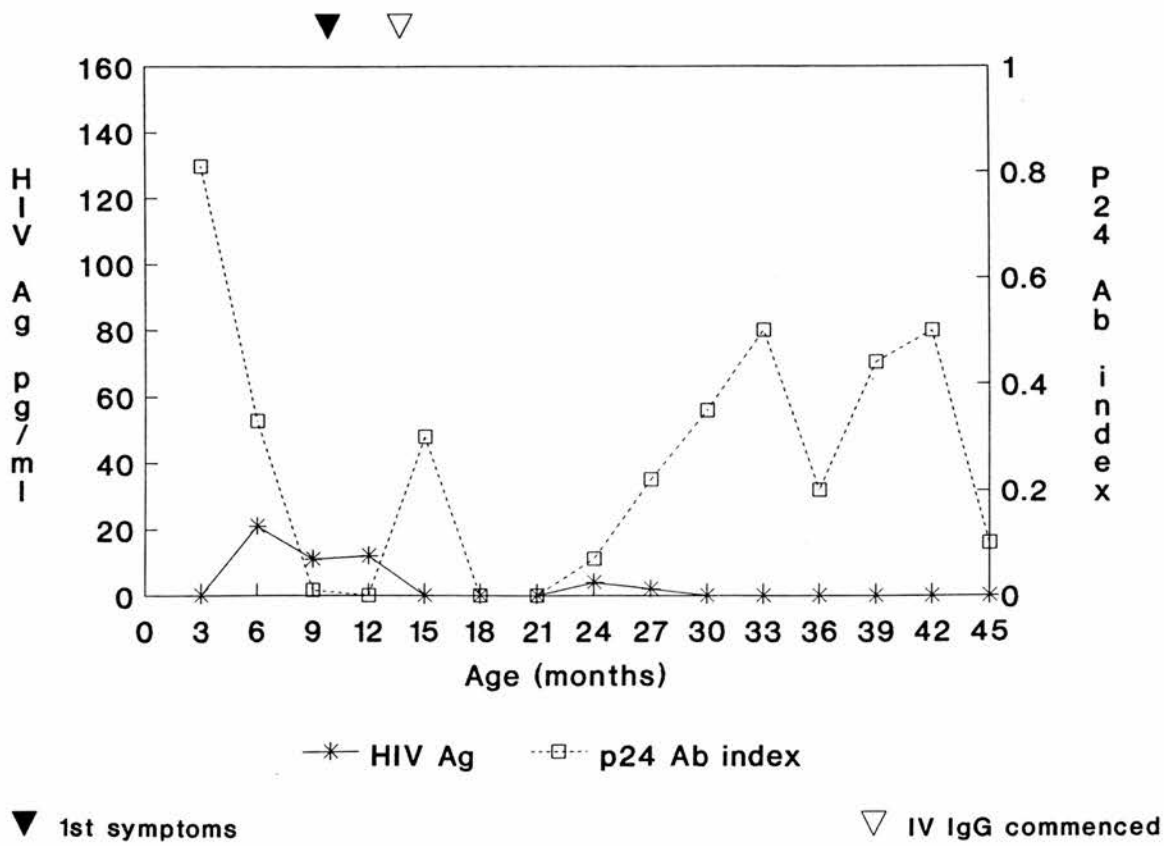

Figure 1. HIV Ag and p24 Ab levels in case 9, from 3 to 45 months.

ts,[7] and continued until death in case 1, and as as supplies lasted in case 5. Little is known t the role of host immunity in HIV infection, but IV antibody has a protective role then passive specific immunization may be beneficial once oral immunity becomes defective. If HIV Ag itself is a direct cause of symptoms or progression in HIV infection, a reduction of HIV Ag levels may also be beneficial.[7] We demonstrated that infusion of plasma rich in p24 Ab was temporarily effective in removing circulating HIV Ag. However, HIV Ag reappeared in 2 weeks. Further studies are required

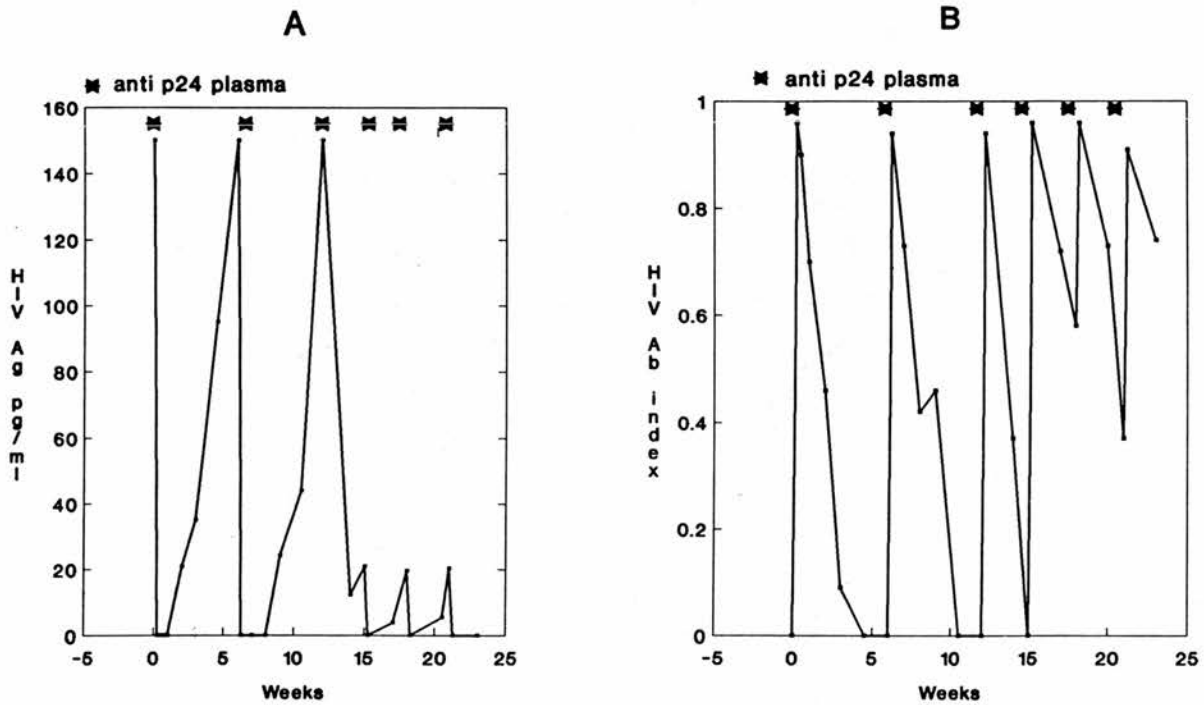

Figure 2. HIV Ag (A) and p24 Ab (B) levels in case 5 during therapy with plasma rich in p24 Ab, starting at time 0.

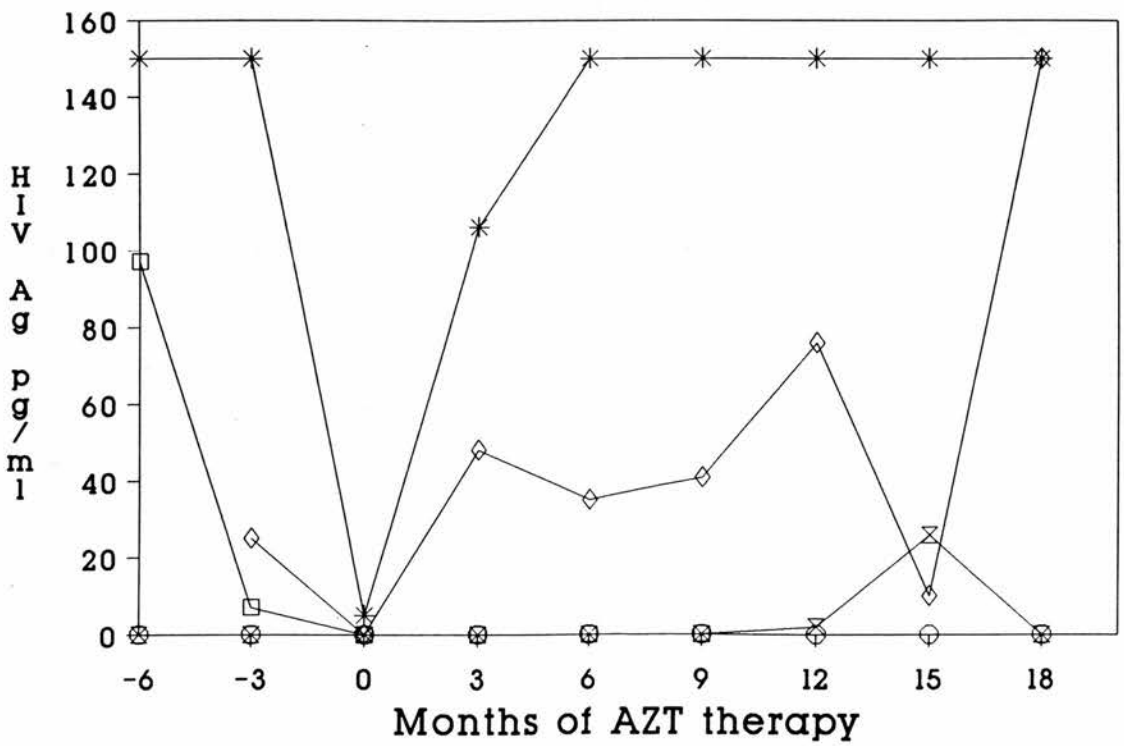

Figure 3. HIV Ag levels before and after zidovudine therapy, commenced at time 0.

mine the dosage, volume, and frequency of tration necessary for sustained antigen sup-, before clinical effects can be evaluated.
patients all commenced treatment with oral ine when HIV Ag levels were low or nega- e were, therefore, unable to confirm previ- eported observations that the levels of HIV following therapy with zidovudine.[5,11,12] r, despite a raised mean corpuscular volume of the red blood cell, suggesting adequate compliance and absorption of the drug, HIV ame positive in two children and persisted n therapy, and reappeared transiently in a wo. This suggests either that viral suppres- y be incomplete or that there is development ance to zidovudine. We were unable to dem- any consistent increase in p24 Ab antibody g therapy with zidovudine.[11] Therapeutic tion did not alter the differences in prognosis d between groups 1 and 2.
onclude that HIV antigenemia may precede ns in HIV-infected children, and thus aid agnosis. High levels of HIV Ag and loss of are poor prognostic signs, irrespective of clinical status, or therapeutic intervention.

owledgments

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Virus infections of the respiratory tract in HIV-infected children

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Summary

In order to determine whether the rates of respiratory viral infection and the severity of respiratory symptoms in HIV-infected children were higher than those in non-infected children, nose and throat swabs for viral isolation were taken at 3-month intervals during the first 2 years of life from 50 children born to HIV-infected women. Similar samples were obtained during the first year of life from 19 control children born to HIV seronegative mothers. Of the 50 children, five proved to be HIV-infected while 45 were presumed to be uninfected. HIV-infected children had significantly more respiratory symptoms and a higher proportion of samples from which viruses were isolated than the non-HIV-infected children. Also, more infected episodes required admission to hospital in the HIV-infected group. There was no such difference between the non-HIV-infected and the control children. Three HIV-infected children received intravenous immunoglobulin therapy. Among these the proportion of positive samples for viral isolation was greater before than after treatment began. These results suggest that HIV-infected children are more susceptible to recurrent viral infection and that passive immunotherapy may be of benefit to such children.

Introduction

The susceptibility of HIV-infected children to respiratory infections caused by bacteria and other opportunistic organisms is well-recorded,[1–3] but the natural history of respiratory infections due to viruses in such children has received little attention. We therefore undertook a prospective study of HIV-infected children in order to determine whether the rates of respiratory viral isolation and the severity of respiratory symptoms in HIV-infected were higher than in non-HIV-infected children using as controls children from the same socio-economic group.

Patients and methods

All the children were among those participating in the Edinburgh HIV Perinatal Transmission Study.[4,5] Fifty children born to HIV seropositive mothers below the age of 2 years were studied prospectively. Five (group A)

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proved to be infected with HIV.[6] One died at 4 months, the remaining four being aged 33–71 months. Three of these children were treated with intravenous immunoglobulin (IV IgG), 200 mg/kg every 3 weeks as previously described.[7] Another group of 45 children (group B) were considered not to be infected with HIV since they were clinically well and repeatedly HIV antibody and antigen negative. As an additional control group, 19 children whose fathers were HIV seropositive but whose mothers were HIV seronegative were also investigated for a period of 1 year (group C), thereafter declining further participation.

All children in the study were seen at 3 monthly intervals and symptoms suggestive of respiratory infection since the previous visit were recorded. Children were examined for signs of respiratory infection. Nose and throat swabs were taken and placed together in viral transport medium. One set of swabs was taken at each visit but results of specimens taken during admission to hospital were not included in this study. Results were analysed by means of the χ^2 test for statistical significance.

Virological methods

Nose and throat swabs taken from each child were combined in a single vial of viral transport medium containing antibiotics to suppress bacterial growth as well as fetal calf serum to stabilise the virus. The samples were transported to the laboratory within 2 h. If any delay was anticipated, swabs in transport medium were stored at 4 °C before transport. In the laboratory, cells collected on the swabs were resuspended in a small volume of transport medium before they were inoculated into cultures of primary baboon kidney, human epithelial cells (HEp2) and human fibroblasts (MRC5). The inoculated cells were incubated at 36 °C and examined twice weekly for evidence of a cytopathic effect. Further confirmatory tests were made on cells affected. Influenza, parainfluenza, respiratory syncytial (RS), measles, and herpes simplex viruses were confirmed by means of monoclonal or polyclonal antibodies in a fluorescent antibody test.[8] Adenovirus, Coxsackie and Echo viruses were identified by neutralisation tests.[9] Rhinovirus was identified by acid stability testing. Cytomegalovirus (CMV) was identified by its characteristic cytopathic effect on MRC5 cells.

Results

A total of 301 samples was collected over a period of 24 months. Results are summarised in Table I. Although a few opportunities for sampling were missed, these did not differ between groups. Group A had significantly more respiratory infections and more samples positive for viral isolation than group B. Over the first year, however, groups B and C did not differ significantly (Table II).

The viruses isolated are listed in Table III. There was no significant difference among the three groups in the proportion of positive virus isolations associated with symptoms. Symptoms usually accompanied infections with influenza A, parainfluenza, RS and adenovirus infections, whereas isolation of CMV or Coxsackie virus was more often asymptomatic. Six of the 10 proven

Table I *Episodes of respiratory infection and virus isolation in HIV-infected and non-infected children in the first 2 years of life*

Group	A	B	*P**
Number of patients	5	45	
Number of samples	30	220	
Age range (mean, median)	3–24 (14·5, 15)	3–24 (14·0, 15)	N.S.
Number of missed sampling opportunities (%)	4/34 (11·8)	22/242 (9·1)	N.S.
Number of symptomatic episodes of respiratory infection	14	41	< 0·01
Number of positive virus isolations	10	32	< 0·05

* According to the χ^2 test.
N.S., Not significant.

Table II *Episodes of respiratory infection and virus isolation in the two control groups of HIV-non-infected children in the first year of life*

Group	B	C	*P*
Number of patients	30	19	
Number of samples	104	51	
Age range (mean, median)	3–12 (7·5, 9)	3–12 (7·9, 9)	N.S.
Number of missed sampling opportunities (%)	11/115 (9·6%)	6/57 (10·5%)	N.S.
Number of symptomatic episodes	13	5	N.S.
Number of positive virus isolations	15	8	N.S.

Table III *Virus isolations from HIV-infected and non-infected children in the first 2 years of life. Comparison of symptomatic and asymptomatic children*

	Symptomatic		Asymptomatic		
Virus	A	B	A	B	Total
Adenovirus	2	2	0	1	5
Influenza	0	3	0	0	3
Parainfluenza	2	2	0	1	5
Respiratory syncytial	0	3	0	1	4
Rhinovirus	1	4	0	3	8
Cytomegalovirus	3	1	2	5	11
Herpes simplex I	0	1	0	1	2
Coxsackie B	0	0	0	2	2
Measles	0	1	0	0	1
Echovirus	0	0	0	1	1
Total	8	17	2	15	42

viral infections in group A necessitated admission to hospital, compared with 9/32 in group B, a difference which was significant ($P < 0{\cdot}05$). All three children with influenza A infection were admitted to hospital. Others were infected with RSV (three in group B), parainfluenza (two in group A, were in group B), adenovirus (two in group A, one in group B) and measles (one in group B). None of the children required assisted ventilation or specific antiviral therapy. Although upper respiratory tract symptoms sometimes accompanied isolation of CMV, CMV pneumonitis was not evident.

The three children from group A who suffered from recurrent infections were given infusions of intravenous immunoglobulin (IV IgG) as prophylaxis against recurrent bacterial infections. In these children, the proportion of positive virus isolation specimens before treatment began was 7/11, compared with 3/13 subsequently, a decrease which also was significant ($P < 0{\cdot}05$). None of the children was treated with zidovudine during this time.

In one child in group B, influenza A infection was associated with a profound drop in the CD4+ lymphocyte count from $2{\cdot}04 \times 10^9$/ml–$0{\cdot}27 \times 10^9$/ml and in the T4/T8 cell ratio form 1·9–0·5. These returned to their previous values on recovery from the illness. We therefore analysed the effects of respiratory symptoms and viral isolation on mean T4 cell counts for groups A and B. No significant difference in mean values was observed between samples taken when the children were asymptomatic and when they had symptoms or a virus was isolated for either group, although the mean was lower in all infected children ($0{\cdot}99 \times 10^9$/l in group A and $2{\cdot}69 \times 10^9$/l in group B). Similarly, no change was seen in CD8+ lymphocyte concentrations.

Discussion

The susceptibility of HIV-infected children to bacterial respiratory infection is well-known,[1] and coincides with a deficiency of antibody. Since HIV-infected children also have a cellular immunodeficiency, they might be expected to be more susceptible to acute viral infections but only two case reports supporting this suggestion have so far been published.[10, 11]

Despite the smaller number of children proving to be HIV-infected among those we studied (5/50) than we had anticipated,[12, 13] we found a significantly increased rate of respiratory viral isolation in the first 2 years of life compared with appropriate controls. This may be due either to HIV infection causing greater susceptibility after exposure to viruses other than HIV, or to a greater virus load and more prolonged excretion during any given episode, so making viral isolation on sampling at intervals more likely. We have not seen the chronic excretion of agents such as parainfluenza virus, which has been reported by others.[11] Moreover, it was perhaps fortuitous that our HIV-infected children escaped RSV when infections were widely prevalent in the community, since prolonged viral carriage and a mortality rate as high as 20% in severely immunocompromised HIV-infected children has been reported.[10]

In our study, we used two different control groups of children who had the same social background as the HIV-infected children. We have thereby demonstrated that children born to HIV seropositive mothers who are not HIV-infected do not differ in their rates of respiratory infection from those not

exposed to the risk of HIV infection. In all three groups, poor housing, overcrowding, and parental smoking habits may have contributed to a higher than average morbidity in this respect.

The finding of fewer positive viral isolates in HIV-infected children following IV IgG therapy should be interpreted with caution since data are available on only three children. One possible explanation could be that children outgrow susceptibility to viral infection after the first year of life but this is not borne out by the rates in group B children which were constant between the 2 years. Immunoglobulin therapy has been shown to prevent Echovirus infection,[14] reduce the shedding of RSV,[15] and may have beneficial effects in CMV infection,[16] suggesting that the reduction of viral isolates may have been a beneficial side-effect of IV IgG therapy.

We conclude that respiratory viral infections arise more often in HIV-infected children. Given the ubiquitous nature of these agents and the lack of available specific therapy for treatment or prophylaxis, further studies are required in larger groups of HIV-infected children to assess the incidence of recurrent viral infections and their influence on the progression of HIV disease together with the role of immunotherapy such as the early use of IV IgG.

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function in the process leading to the initiation of parturition in human beings, possibly by shifting the balance of the production of eicosanoids in favour of those derived from n-3 rather than n-6 fatty acids.[5,8] Biochemical and physiological studies are needed to clarify how and at what concentrations dietary n-3 fatty acids could affect formation of eicosanoids in human pregnancy and to compare the biological properties in the uteroplacental system of those derived from n-3 and n-6 fatty acids. Our findings suggest an easy and cheap intervention to prevent preterm delivery. However, prolongation of gestation by fish oil may not result in reduced frequencies of the complications associated with preterm delivery;[1] whether it will or not depends on the extent to which preterm delivery is a necessary link in the causal chain leading to the complications. Furthermore, fish-oil supplementation could lead to more post-term deliveries, which are also associated with complications.[2,3] A carefully monitored, controlled trial of fish oil to women at high risk of preterm delivery seems justified, and a multicentre trial is in progress.[18-20]

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Risk factors for mother-to-child transmission of HIV-1

EUROPEAN COLLABORATIVE STUDY

Children born to women known to be infected with human immunodeficiency virus type 1 (HIV-1) before delivery were followed prospectively from birth in nineteen European centres. This analysis, encompassing the period end-December, 1984, to beginning-August, 1991, focuses on risk factors for mother-to-child transmission of HIV-1 infection.

Rate of vertical transmission, based on 721 children born to 701 mothers more than 18 months before the time of analysis, was 14·4% (95% CI 12·0–17·1%). Transmission was associated with maternal p24-antigenaemia and a CD4 count of less than 700/μl. In a multivariate analysis, odds ratios of transmission were: 2·25 (95% CI 0·97–5·23) in breastfed children *vs* never-breastfed children; 3·80 (1·62–8·91) in children born before 34 weeks' gestation; and 0·56 (0·30–1·04) in children delivered by caesarean section. Transmission was higher with vaginal deliveries in which episiotomy, scalp electrodes, forceps, or vacuum extractors were used, but only in centres where these procedures were not routine.

On the basis of these results, HIV-infected women contemplating pregnancy should be counselled according to their immunological findings and, if they have p24-antigenaemia or a low CD4 count, warned of an increased risk of viral transmission. Caesarean deliveries may have a protective effect, although it is premature to recommend routine operative delivery. The mechanism for the higher infection rate in children born before 34 weeks' gestation is unclear, but could reflect inadequate passive or active immunity at that age, combined with substantial transmission during labour or delivery. The balance of evidence suggests that mothers with established infection can transmit HIV infection through breastmilk, although the relative importance of this route remains to be defined.

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Introduction

Reported rates of transmission of human immunodeficiency virus type 1 (HIV-1) infection from mother to child range from 7 to 39%.[1-8] It is unclear to what extent this variation is due to methodological differences or to different distributions of risk factors in the populations studied. There is some evidence that stage of maternal HIV infection, as indicated by clinical and immunological status, is associated with increased transmission.[2,4,9,10] Some researchers[11,12] have suggested that most HIV infection is acquired around the time of delivery. Mode of delivery has not been shown to play a part in HIV transmission,[1,13] but there is no information about the role of obstetric procedures or events in the intrapartum period. Although breastfeeding during primary infection poses a risk,[14] the additional risk of breastfeeding in established maternal infection is poorly quantified.[13,15]

In this report we analyse data collected by participants in the European Collaborative Study from end-December, 1984, to beginning-August, 1991.[5] Our aim was to investigate the role of factors that may be associated with vertical transmission of HIV-1 infection—for example, clinical and immunological status of the mother during pregnancy, length of gestation, mode of delivery, and breastfeeding.

Methods

Mothers and infants

In ten participating centres infants born to women known to be HIV-1 seropositive at or before delivery were enrolled at birth and followed-up regularly according to a standard protocol.[5,16] At the time of birth, we obtained information about maternal clinical status, mode of delivery, length of gestation, and birthweight for these children, and we sought further information about maternal immunological variables and obstetric procedures from the obstetricians in these centres. In 1991, obstetricians in an additional nine centres provided similar information which they had already collected systematically on all HIV-1 seropositive mothers who were known to be infected before delivery, and on the subsequent infection status of the children.

All infants born 18 months or more before the date of analysis are included. HIV-1 infection in the child was defined as: AIDS;[17] HIV-related death; persistence of antibody beyond 18 months; or detection of virus or p24-antigenaemia in at least two samples. 4 children who became seronegative but who were repeatedly virus positive were excluded from the analysis.[5] The remaining seronegative children are judged to be uninfected.

All laboratory assessments were carried out locally; lymphocyte subsets were determined by immunofluorescence with cytofluorometric readout.[18] The presence of p24-antigenaemia was determined by commercially available enzyme-linked immunosorbent assays (Abbott, Du Pont, Pasteur, Organon) or, in one centre, by an in-house system.[19] The result closest to delivery was used in the analysis provided the maternal sample was taken during pregnancy or less than 1 month after delivery. 86% of the p24 antigen tests were done during the third trimester or at the time of delivery, as were 76% of the lymphocyte subset estimations. CD4 counts were divided into three groups of approximately equal numbers by means of cut-off values of 400 and 700 per μl. The CD4/CD8 ratio was divided similarly, the values being 0·6 and 0·9. This approach was found to be more appropriate than conventional categories such as CD4 counts of less than 200/μl since only 9 women in this largely symptom-free cohort fell into this category.

Women were included as HIV-symptomatic (Centers for Disease Control [CDC] stage III or IVA) if any HIV-related symptom or sign was recorded by the clinician on two or more occasions up to 3 months post partum. AIDS was defined according to the CDC classification,[20] and a mother was regarded as having AIDS if the diagnosis was made no later than 1 year post partum, to allow for increased infectiousness before onset of AIDS.[21]

TABLE I—RATE OF TRANSMISSION BY SELECTED RISK FACTORS

—	Mother-child pairs		Infected children	
	Total	Children with known infection status	No (%)	95% CI
All children	831	721	104 (14)	12–17
Maternal characteristics				
Intravenous drug use				
Ever	654	571	75 (13)‡	10–16
Never	174	148	28 (19)	13–26
Parity				
Primiparous	489	437	62 (14)	11–18
Multiparous	323	269	42 (16)	12–20
Race*				
White	769	666	93 (14)	11–17
Black	39	32	6 (19)	8–35
Asian or other	17	17	5 (29)	12–54
Maternal clinical/ immunological findings				
Clinical progression†				
None	685	602	84 (14)	11–17
Signs, no AIDS	100	83	14 (17)	10–26
AIDS	13	13	4 (31)	11–59
p24-antigenaemia				
Negative	336	288	30 (10)¶	7–14
Positive	45	42	12 (29)	16–44
CD4 count/μl†				
<400	83	72	14 (19)§	11–30
400–699	90	80	18 (22)	14–33
≥700	85	78	5 (6)	2–14
CD4/CD8 ratio†				
<0·60	106	88	21 (24)§	16–34
0·60–0·89	78	73	9 (12)	6–21
≥0·90	67	63	7 (11)	5–21
Delivery and breast feeding				
Mode of delivery*				
Vaginal	614	547	85 (16)	13–19
Elective caesarean	155	127	16 (13)	8–19
Emergency caesarean	60	45	3 (7)	2–17
Gestational age				
≥34 weeks	774	678	93 (14)¶	11–17
≤33 weeks	43	33	11 (33)	19–51
Child ever breast fed				
No	790	683	93 (14)§	11–17
Yes	38	36	11 (31)	17–47

Numbers do not always add up to total because of missing data.
*Significance assessed by test for heterogeneity; †Significance assessed by test for trend; ‡$p < 0·1$; §$p < 0·05$; ¶$p < 0·01$.

Zidovudine was not prescribed during pregnancy in the participating centres, but 4 women, all symptomatic, were already receiving the drug when their pregnancy was diagnosed.

Gestational age was recorded to the nearest completed week and assessed by ultrasound when available or otherwise by date of last menstrual period or clinical judgment.

Statistical analysis

Variation between centres was assessed by unconditional logistic regression, after allowance for the risk factors listed in table II. Conditional (upon centre) logistic regression was used to estimate independent effects of selected risk factors on transmission (tables II and IV). Exact mid-p CIs were calculated for proportions.

Results

Rates of transmission

Between end-December, 1984, and beginning-August, 1991, 1005 children had been enrolled from the nineteen centres, of whom 831 were born at least 18 months before the date of analysis. HIV-infection status was determined in 721 (87%) children of 701 mothers. Of the remaining 110 (13%) children whose infection status was not known, 13 had died of causes not thought to be related to HIV infection, 45 were lost to follow-up within 1 month of birth, and 52 were either lost to follow-up after that age or had not been seen at the appropriate scheduled times. 104 children

TABLE II—MULTIVARIATE ANALYSIS OF RISK FACTORS FOR TRANSMISSION

	Odds ratio				
—	Crude*	Adjusted†	Adjusted‡	95% CI	p‡
Mode of delivery					
Vaginal	1·00	..	..	..	..
Elective caesarean	0·78	0·74	0·65	0·32–1·31	0·23
Emergency caesarean	0·39	0·34	0·37	0·11–1·25	0·11
Gestational age					
≥ 34 weeks	1·00	..	..	..	..
≤ 33 weeks	3·15	3·07	3·80	1·62–8·91	0·002
Child ever breast fed					
No	1·00	..	..	..	..
Yes	2·79	2·22	2·25	0·97–5·23	0·06
Intravenous drug use					
Ever	1·00	..	..	..	..
Never	1·54	1·45	1·28	0·73–2·26	0·39

Analysis based on 664 children (101 infected).
*Derived from table I; †for centre; ‡for centre, mode of delivery, gestational age, breastfed, clinical progression, IVDU, parity, and race.

were infected, of whom 34 had been diagnosed with AIDS and 1 had died with persistent oral candidosis, a sign previously found to be predictive of imminent onset of AIDS. Rate of vertical transmission was 14·4% (95% CI 12·0–17·1%). There was no trend over time ($\chi^2_{trend} = 0·80$, $p = 0·37$), nor was there significant variation between the centres ($\chi^2 = 22·30$, adjusted for risk factors, 18 df, $p = 0·22$). In particular, there was no difference in transmission rate between the ten original centres and the nine additional ones ($\chi^2 = 0·47$, 1 df, $p = 0·49$).

Table I shows the number of children with known infection status and rate of transmission by selected risk factors. Although ascertainment of infection status was more complete for children of mothers with high CD4/CD8 ratios, for children delivered vaginally, and for those born after 33 weeks' gestation, these differences are unlikely to have biased the results substantially.

554 (79%) women had acquired HIV infection through intravenous drug use (IVDU); of the 147 women without a history of IVDU, 62 (42%) had an IVDU partner and 25 (17%) came from, or had a partner who came from, a high-risk area. Rate of transmission was lower in women with a history of IVDU, largely due to a low rate (8%) in former drug users. Vertical transmission was not significantly associated with parity, race (table I), or maternal age at delivery.

The risk of infection was greatest (31%) for infants born to the 13 women with AIDS, but less advanced HIV-related manifestations were not predictive or transmission. Rate of transmission rose sharply for a CD4 count below 700/μl or for a CD4/CD8 ratio below 0·6, and was strongly associated with p24-antigenaemia. 4 women were classified as CDC stage I (acute primary infection) at the time of delivery; all 4 were p24 antigen negative and 2 of the children were infected.

Most (76%) children were delivered vaginally, and the crude rate of transmission in this group was similar to that of children delivered by elective caesarean section. The lower rate of transmission in children delivered by emergency caesarean section than by an elective operation may be a chance finding ($\chi^2 = 0·66$, 1 df, $p = 0·42$).

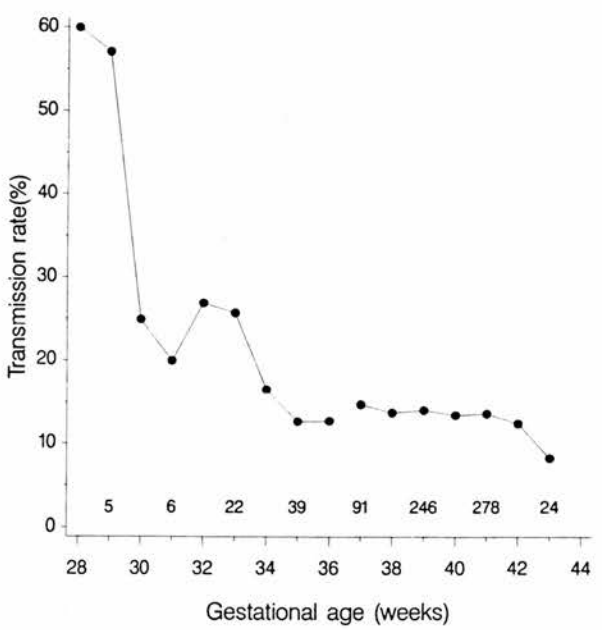
The relation between transmission rate and gestational age was non-linear, with a higher risk of infection in children born before 34 weeks but little further reduction in risk beyond this age (see figure). The 33 children born before 34 weeks' gestation had a 33% infection rate *vs* 14% for children born thereafter (table I). Of the 11 infants delivered by caesarean section before 34 weeks, 3 (27%) were infected, which was not significantly different from the rate in infants delivered vaginally (8/22, 36%).

When uninfected children were taken as a standard, and with adjustment for maternal IVDU, birthweight of the infected children was appropriate for gestational age, both in the cohort as a whole and in the group of children born before 34 weeks' gestation.

In all centres, HIV-positive women were discouraged from breastfeeding. 36 (5%) mothers nevertheless chose to breastfeed, although duration of breastfeeding tended to be short (median 4 weeks). Rate of vertical transmission was significantly higher in breastfed (31%, table I) than in never-breastfed infants (14%), but there was no association with duration of breastfeeding. 6 of 17 (35%) children breastfed for less than 4 weeks were infected *vs* 5 of 19 (26%) breastfed for longer.

Risk factors for vertical transmission: multivariate analysis

Multivariate analysis was done to assess the effect of selected risk factors while controlling for other factors (table II). Adjustment for centre effectively produced a stratified analysis and additional adjustment for other risk factors allowed for associations between risk factors within centre. The association between mode of delivery and transmission was strengthened, largely as a consequence of a higher proportion of symptomatic women in the elective caesarean section group. Odds of infection in children delivered by elective caesarean section was 0·65 times that of children delivered vaginally, or 0·56 (95% CI 0·30–1·04, $p = 0·07$) times if elective and emergency caesarean sections are combined (table II). IVDU was a risk factor for prematurity; after allowance for this fact, children born before 34 weeks' gestation had a nearly four-fold higher odds of infection than those born after 33 weeks. Breastfeeding was more more common in some centres; controlling for centre weakened the association because the rate of transmission among non-breastfeeders in these



Transmission rate by gestational age.

Figure has been smoothed by plotting 3-point averages—eg, value at 36 weeks is observed transmission rate for 35–37 weeks. Numbers born by gestational age are shown in 2-week intervals.

TABLE III—MATERNAL CLINICAL AND IMMUNOLOGICAL VARIABLES BY SELECTED RISK FACTORS

—	CD4/μl		p24 antigen		AIDS or HIV signs	
	No	GM	No	No (%) positive	No	No (%) affected
*Mode of delivery**						
Vaginal	172	520	249	28 (11)	533	62 (12)¶
Elective caesarean	45	520	57	11 (19)	120	29 (24)
Emergency caesarean	13	500	22	1 (5)	43	5 (12)
Gestational age						
≥34 weeks	209	680	308	34 (11)§	658	87 (13)‡
≤33 weeks	15	580	18	6 (33)	30	8 (27)
Child ever breast fed						
No	225	520	319	38 (12)	660	94 (14)
Yes	5	530	9	2 (22)	36	2 (6)
Intravenous drug use						
Ever	174	520	258	31 (12)	551	75 (14)
Never	56	500	70	9 (13)	145	21 (14)
Clinical progression†						
None	172	550¶	265	26 (10)¶	..	..
Signs, no AIDS	53	410	55	12 (22)	..	..
AIDS	2	250	5	2 (40)	..	..
p24-antigenaemia						
Negative	179	540§	..	..	..	..
Positive	23	400	..	..	..	..

GM = geometric mean.
*Significance assessed by tests for heterogeneity.
†Significance assessed by tests for trend.
‡$p < 0.1$; §$p < 0.05$; ¶$p < 0.01$.

centres was higher than among non-breastfeeders in general. Infants of black mothers (6) and of non-IVDU mothers (17) and those delivered vaginally (31) were over-represented among breastfeeders, but adjustment for these and other risk factors made little difference to the estimated effect of breastfeeding. The negative association between IVDU and transmission was further reduced after adjustment for centre, mode of delivery, gestational age, clinical progression, parity, and race.

Maternal clinical and immunological markers

The model shown in table II did not include maternal p24-antigenaemia or CD4 count because this information was routinely collected as part of the protocol only in some centres after a centre-specific date. Table III shows the association between selected risk factors and maternal clinical and immunological variables. Too few breastfeeding mothers had p24 antigen tests or CD4 counts recorded to exclude the possibility that they were more infectious than other mothers. IVDU women did not differ from women without a history of IVDU. Clinical signs attributed to HIV-1 infection and p24-antigenaemia were more frequent in women who had an elective caesarean section and in the mothers of children born before 34 weeks' gestation. The significantly lower CD4 counts and higher rate of p24 antigenaemia in the symptomatic women suggest that maternal signs were related to HIV infection.

TABLE IV—RISK OF TRANSMISSION ASSOCIATED WITH PROCEDURES USED IN VAGINAL DELIVERIES

—	Children with known infection status	Infected children	Odds ratio		
		No (%)	Crude	Within centre	p*
Episiotomy†					
No	50	6 (12)	1·00		
Yes	157	29 (18)	1·66	5·59	0·02
Scalp electrodes					
No	382	59 (15)	1·00		
Yes	91	13 (14)	0·91	2·35	0·05
Instrumental					
No	521	79 (15)	1·00		
Yes	26	6 (23)	1·68	2·70	0·07

*p values are "exact" one-sided.
†Information on use of episiotomy collected only in later stages of the study.

Procedures/interventions during vaginal delivery

Rate of transmission in children born by vaginal delivery was similar irrespective of the use of episiotomy, scalp electrodes, or instruments (table IV). However, there was wide variation between centres in the use of these procedures and within-centre comparisons suggested that such interventions were associated with an increased risk of transmission in centres where they were not routinely adopted. Further allowance for the risk factors in table II did not alter this finding.

Discussion

The lack of significant variation in transmission rates between centres suggests that there are no major unrecognised local risk factors. It has been suggested that IVDU could adversely affect the immune system, thereby increasing the risk of vertical transmission of HIV infection. However, in this study IVDU was not associated with adverse immunological findings or with higher rates of transmission. Similarly, neither parity nor age of mother at the time of the delivery was associated with transmission.

Previous reports of an association between maternal CD4 count and risk of vertical transmission have been contradictory,[2,4,22] and p24-antigenaemia has been linked to an increased rate of transmission in a small cohort of mother/child pairs.[22] Our study establishes that a low CD4 count and p24-antigenaemia are predicitive of vertical transmission, whereas non-specific clinical manifestations of HIV infection are not. CD4 counts and p24-antigenaemia can be used as surrogate markers for viral load, although infectiousness is best estimated by quantification of virus in cells and plasma.[23] Our findings should provide an impetus for studies of antiretroviral therapy or anti-HIV immunoglobulin[24] during pregnancy, although the effect of these drugs on the unborn child will need to be considered carefully.

HIV seropositive women contemplating pregnancy or considering termination of pregnancy should be counselled on the basis of both laboratory and clinical investigations. Women who have p24-antigenaemia or a low CD4 count should be advised that they are about twice as likely to transmit the virus to their offspring. Risk of vertical transmission seems to increase for CD4 counts below 700/μl, higher than the standard concentrations currently used for initiating antiretroviral or prophylactic treatment in adults.[25] Most antigen and CD4 testing was done in the third trimester of pregnancy. Although a low CD4 count early in or before pregnancy is likely to be followed by a low count later in pregnancy,[26] the predictive value of early antigenaemia remains to be established.

The high frequency of HIV infection in children born before 34 weeks' gestation could be explained in several ways. First, HIV infection in utero could affect fetal development and lead to premature delivery. By analogy with other congenital infections,[27] it would then be expected that infected children would be small-for-dates as well as premature, but this was not observed. Second, women who are more likely to be infectious, as indicated by antigenaemia or AIDS, may be more likely to deliver before 34 weeks. Third, concurrent infections, especially those of the genital

tract, may increase both the risk of premature delivery[28] and the risk of transmission of HIV infection. The latter could arise through chorioamnionitis[29] or by attracting HIV-infected lymphocytes to the birth canal. We have no data on the presence or absence of genital tract infections and cannot test this hypothesis. These suggestions would accord with a continued decrease in the rate of transmission with gestational age. Finally, infants born before 34 weeks may be more susceptible to intrapartum HIV-1 infection because of diminished immunocompetence and, possibly, low concentrations of passively acquired antibodies. Goedert et al[4] reported a high rate of transmission in children born before 38 weeks' gestation, which they specifically attributed to low concentrations of maternal gp-120 antibodies, but the protective effect of gp-120 antibodies in the transmission of HIV was not supported by results from the New York collaborative study.[30] For prematurity to be risk factor for acquisition of infection, a substantial proportion of infants should acquire infection during labour or delivery. Our data showed a levelling off in rate of transmission after 34 weeks' gestation, which would accord with rapid transfer of maternal antibodies late in pregnancy and increased susceptibility of very premature infants.

Scalp electrodes, episiotomy, and instrumental vaginal deliveries were associated with subsequent infection in infants only in centres where these procedures were not routine, which suggests that such factors per se do not pose a genuine risk. A more plausible explanation is that in these centres interventions are used selectively in more complex deliveries. Although transmission can occur early in pregnancy,[31] the association with scalp electrodes &c lends further support to the view that a substantial proportion of infection is acquired around the time of delivery.[11] The report by Goedert et al[12] that the first-born twin had a significantly higher risk of being infected than the second-born twin specifically implicated transmission in the birth canal. Transmission around the time of delivery could occur through mixing of maternal and fetal blood,[32] or via ingestion of HIV-containing body fluids.

Although the relative importance of transmission at delivery is becoming apparent, prospective studies[1,12,13] have shown similar rates of transmission in children delivered vaginally and by caesarean section. In our study, there was some evidence of a protective effect of operative delivery but the result was not statistically significant. If one assumes a 15% risk of transmission by vaginal delivery, the results from our model predict that the risk by caesarean section would be 9% (95% CI 5–16%). Thus the data accord both with section reducing the risk of transmission approximately three-fold (from 15% to 5%) and with the procedure offering no protective effect. However, some obstetricians were more likely to carry out an elective caesarean section on women who were antigenaemic or HIV-symptomatic, and there may be other biases of which we are unaware. Available evidence is therefore not sufficiently clear to recommend routine caesarean section, and randomised controlled trials are warranted to evaluate the effect of mode of delivery on the risk of transmission.

Van de Perre et al[14] found a high risk of transmission (at least 4 of 11) of HIV infection through breastfeeding in primary infection after delivery. Since viraemia is common shortly after infection,[33,34] and since these children would not possess passively acquired maternal HIV-antibodies, the relevance of this finding for mothers with established infection is unclear. World Health Organisation guidelines[35] discuss the need to balance the benefits of breastfeeding by HIV-infected women against the additional risk of transmission through breastmilk. Prospective studies have failed to recruit both breastfed and exclusively bottlefed infants in sufficient numbers: Blanche et al[1] and Ryder et al[15] reported higher rates of transmission in breastfed children, whereas Hutto et al[13] reported a marginally smaller risk among breastfeeders. We found a two-fold increase in the risk of infection among breastfed children, although the number of children in this group was small. The balance of evidence suggests that mothers with established infection can transmit HIV infection through breastmilk, although the relative importance of this route remains to be quantified.

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Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction

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The cause of sarcoidosis is unknown. However, the histological similarity between the disorder and tuberculosis suggests that mycobacteria might contribute to the pathogenesis of sarcoidosis. We have used the polymerase chain reaction (PCR) to detect mycobacterial DNA in clinical samples from patients with sarcoidosis.

104 patients were included in the study (62 referred for possible tuberculosis and 20 for possible sarcoidosis, and 22 control patients who had undergone bronchoscopy for other reasons). Bronchoalveolar lavage samples, bronchial washings, and tissue specimens (1 from each patient) underwent assay by PCR as well as bacteriological, histological, and cytological examination. We used two PCR reactions: in the first the complex-specific insertion sequence IS986/IS6110 was used to specifically detect DNA from *Mycobacterium tuberculosis* complex bacteria; in the second, conserved sequences of the mycobacterial *groEL* gene were used to detect DNA from mycobacteria other than *M tuberculosis*. Th PCR was more sensitive than culture for diagnosis o tuberculosis. However, the false-positive PCR rat for *M tuberculosis* was 9%. *M tuberculosis* DNA wa found in half the sarcoidosis patients, and non tuberculosis mycobacterial DNA in a further 20%.

The findings that a significant proportion of th sarcoidosis patients in this study have mycobacteri in their lungs and that most of these mycobacteri belong to *M tuberculosis* complex suggest a aetiological role for mycobacteria in sarcoidosis.

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